UNIVERSITY OF MICHIGAN
DENTISTRY LIBRARY

36 No. 4

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF
THE INTERNATIONAL ASSOCIATION
FOR DENTAL RESEARCH

Editor
HAMILTON B. G. ROBINSON

Published for
THE INTERNATIONAL
ASSOCIATION FOR
DENTAL RESEARCH

By
THE C. V. MOSBY COMPANY
ST. LOUIS 3, MO.

Copyright © 1957 by The International Association for Dental Research

AUGUST, 1957

Editor's Viewpoint ---------- 493

Effects on Monkeys of Introduction of Hemolytic Streptococci Into Root Canals. Donald R. Kennedy, D.D.S., Tom R. Hamilton, M.D., and Jerome T. Syverton, M.D. (University of Minnesota, Minneapolis, Minn.) ---------- 496

Placental Permeability to Bacterial Toxins. Holmes T. Knighton, D.D.S., Seymour J. Kreshover, D.D.S., M.D., Ph.D., and James A. Hancock, Jr., M.S. (The Medical College of Virginia, Richmond, Va.) 507

Fusiforms in the Infant Mouth. Valerie Hurst, Ph.D. (University of California Medical Center, San Francisco, Calif.) ---------- 513

The Extra-Osseous Innervation of the Gingivae. Dipya Mongkollugsana, M.S., D.M.D., and Linden F. Edwards, Ph.D. (The Ohio State University, Columbus, Ohio) ---------- 516

The Relation of Salivary Sodium, Potassium, Solids and Ash Concentration to Dental Caries Experience in Children, 5 to 6 and 12 to 14 Years of Age. I. Zipkin, F. A. Bullock, and N. Mantel (National Institute of Dental Research, National Institutes of Health, Bethesda, Md.) ---------- 525

Early Administration of Various Carbohydrates and Subsequent Dental Caries in the Rat. R. R. Steinman and M. I. Haley (College of Medical Evangelists, School of Dentistry, Loma Linda, Calif.) ------- 533

(*Contents continued on inside back cover*)

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

THE JOURNAL OF DENTAL RESEARCH

Editor—Hamilton B. G. Robinson (Ohio State University). *Business Manager*—J. Frank Hall (Indiana University). *Circulation Manager*—Paul E. Boyle (Western Reserve University).

Assistants to the Editor—Paul C. Kitchin and J. B. Brown.

Publication Committee—H. B. G. Robinson, chairman; J. Frank Hall, Paul E. Boyle, Reidar F. Sognnaes, Ned B. Williams, D. Y. Burrill.

BOARD OF EDITORS

D. A. Afonsky	S. J. Kreshover	J. C. Muhler	J. H. Shaw
G. J. Cox	R. S. Manly	F. J. Orland	W. G. Shafer
S. B. Finn	J. B. Macdonald	W. Pigman	R. F. Sognnaes
M. K. Hine	D. F. Mitchell	T. Rosebury	S. Wah Leung

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES TO THE COUNCIL

Division	*Section*	*Secretary*	*Councillor*
North America	Toronto	R. M. Grainger	G. Nikiforuk
	Winnipeg	E. Roy Bier	H. J. Merkeley
	Ann Arbor	K. Asgar	D. A. Kerr
	Baltimore	L. W. Gruber	E. C. Dobbs
	Birmingham	L. Robinson	J. F. Volker
	Boston	N. C. Turner	R. S. Manly
	Chicago	S. Pearlman	F. J. Orland
	Cleveland	S. W. Chase	T. J. Hill
	Columbus	Steve Kolas	Wm. Lefkowitz
	Detroit		P. C. Lowery
	Houston	D. A. Hagerman	S. S. Arnim
	Indianapolis	H. M. Swenson	W. G. Shafer
	Iowa City	K. E. Wessels	W. J. Simon
	Kansas City	Ben W. Warner	H. L. Myers
	Louisville	R. E. Myers	D. Y. Burrill
	Minnesota	Robert Sausen	W. H. Crawford
	Nebraska	D. A. Keys	D. A. Keys
	New Orleans	H. W. Peterson	H. W. Peterson
	New York	P. L. Johnson	G. Bevelander
	Pacific Northwest	H. W. Fleege	B. O. A. Thomas
	Philadelphia	S. Oliet	L. I. Grossman
	Pittsburgh	J. Nathans	W. F. Swanson
	Richmond	J. J. Salley	H. T. Knighton
	Rochester	F. Brudevold	B. G. Bibby
	San Francisco	S. Epstein	W. C. Fleming
	St. Louis	J. R. Ring	T. Rosebury
	Southern Calif.	L. A. Bavetta	B. Z. Rabinowitch
	Tennessee	J. T. Ginn	O. A. Oliver
	Washington	W. M. Ludwick	D. B. Scott
British		C. H. Tonge	M. A. Rushton
Not belonging to a Division	Denmark	P. O. Pedersen	J. J. Holst
	Helsinki	K. Koski	
	Japan	K. Enoki	
	Johannesburg	J. Staz	
	Mexico	F. R. Leycegui	F. R. Leycegui
	Norway	G. Toverud	
	Palestine	S. Lewin-Epstein	
	Prague	F. Neumann	
	Austria, Vienna Center	E. Zitka	F. Driak
Dental Materials Group		D. B. Mahler	H. D. Coy

OFFICERS OF THE ASSOCIATION

President—Reidar F. Sognnaes (Harvard University), *President-Elect*—Ned B. Williams (University of Pennsylvania), *Vice-President*—Hamilton B. G. Robinson (Ohio State University), *Secretary-Treasurer*—D. Y. Burrill (University of Louisville), *Assistant Secretary-Treasurer*—Edward H. Hatton (Northwestern University), *Editor*—Hamilton B. G. Robinson (Ohio State University).

Vol. 36, No. 4, August, 1957. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription rates: United States and its Possessions $10.00, Students $5.00; Canada, Latin-America, and Spain $10.50, Students $5.50; Other Countries $11.00, Students $6.00. Single copies $2.50 postpaid. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A.

EDITOR'S VIEWPOINT

Kennedy, Hamilton, and Syverton (p. 496) introduced hemolytic streptococci into the root canals of 8 monkeys and studied their reaction in an effort to ascertain the relationship of root canal infection to rheumatic heart disease. Although the bacteria persisted in the root canals, transient bacteremias were produced and significant response of the antistreptolysin 0 titer was observed, no typical cardiac changes were observed. This study does not establish that rheumatic heart disease cannot be effected by dental or periodontal disease because other factors, such as prior heart valve injury, may be important.

The term "placental barrier" is often used to describe the assumed placental protection against fetal invasion by bacteria or their toxins. Knighton, Kreshover, and Hancock (p. 507) tested the effectiveness of the barrier against the exotoxins of 3 organisms in animals. By their methods, they were unable to detect the toxins in fetal blood or tissues although the maternal blood was positive for their presence. A histamine-releaser and a spreading agent did not affect any change in placental transfer. The possibility of the fetus being disturbed as a result of maternal infection acting through the maternal metabolism is not discounted by the authors.

Despite the often repeated claims that anaerobic organisms do not appear in the human mouth until after the teeth erupt, i.e., the gingival crevice appears, Hurst (p. 513) almost invariably isolated gram-negative rods and filaments from the mouths of 2- to 5-month-old babies (63 subjects) and in 58 per cent of 18 edentulous infants subcultured anaerobic colonies of fusiforms. These findings refute the generally accepted concept of the anaerobe-free oral flora of the infant and suggest revision of certain concepts based on the erroneous viewpoint.

Mongkollugsana and Edwards (p. 516) studied the extraosseous innervation of the gingiva by gross dissection. Despite the antiquity of anatomy the information in this particular area is meager. The extraosseous nerves investigated were those, other than the alveolar or dental nerves, which course within the bone and supply the periodontal structures. Because of the lack of agreement on the innervation of these tissues, as tabulated from 5 standard textbooks, the results are significant, especially to the anesthetist.

Zipkin, Bullock, and Mantel (p. 525) used a flame photometer method to determine the salivary sodium and potassium levels of 510 children with available records of past caries experience. No significant difference was found between past caries experience and salivary potassium and sodium levels. Although no sex differences in salivary P and K levels were noted at 5 to 6 years of age, they did become significant at 12 to 16 years of age. It is unfortunate that these salivary studies were tied to past caries experience rather than to activity of the carious process.

From a limited study of small groups of rats, Steinman and Haley (p. 532) found that early feeding of carbohydrates, i.e., feeding during odontogenesis, affected the subsequent rate of tooth decay. This hypothesis has been proposed from other limited studies and deserves a more complete investigation and analysis before it is either accepted or rejected.

The distribution of carious lesions within different teeth has not been reported in any detail. Barr, Diodati, and Stephens (p. 536) conducted clinical and radiographic studies of carious lesions in 583 patients of 13 to 40 years of age. The amount of proximal and pit and fissure attack after 21 years of age was negligible but the

UNIVERSITY OF MICHIGAN LIBRARIES

increment of cervical caries continued progressively to the end of the age period in the study. There was no correlation between the incidence of pit and fissure and proximal cavities. Information of this sort strongly suggests that the process of dental caries may not be a single process but that there may be different types of caries just as there are different types of pneumonia.

McCauley and Frazier (p. 546) report on a careful study of caries experience in 2,520 Baltimore school children selected from 15 schools with white children and 6 with Negro students. The caries experience was similar to that found in a survey made 3 years earlier in 12,000 Baltimore school children. No relationship between caries experience and socioeconomic level was apparent but those children with better oral hygiene indices had significantly less decay than those with low indices. The children at higher socioeconomic levels showed more dental care than those at lower levels except where a vigorous dental care program was being operated by the health department. Since Baltimore fluoridated its water 27 months before this study was instigated, it will serve an interesting base line for investigation into the effect of fluorides on dental caries and on dental treatment.

Wagner and Muhler (p. 552) supplied "naturally' fluoridated waters and "artificially" fluoridated waters at 1 and 8 ppm to growing rats. There were no significant differences in skeletal retention between those receiving the naturally fluoridated waters and those receiving waters fortified by addition of sodium fluoride. Numerical differences in retention were observed but may be related to interference with intestinal absorption by the organic components in the naturally fluoridated waters.

Bixler, Webster, and Muhler (p. 559), from studies of salivariadenectomized female rats, conclude that the effect of removal of the salivary glands on the reproductive organs is not entirely nutritional. The pituitaries of the operated animals showed increased amounts of gonadotrophic hormones (FSH and LH) but apparently their release is inhibited. This complex portion of the hormonal relationship of salivary glands in the rat has been the subject of a series of papers from the authors' laboratory (see below).

Bixler, Webster, and Muhler (p. 566) investigated the effect of testosterone, thyroxin, and cortisone on the salivary glands of hypophysectomized rats. Testosterone and thyroxin, together, prevented the usual salivary gland atrophy observed in hypophysectomized rats but cortisone did not appear to have significant influence. Still more study is necessary in this field before a clear interpretation of the relationship of the adrenal glands and the salivary glands can be justifiably established.

Bixler, Muhler, and Shafer (p. 571) injected graded doses of antithyroid radioactive iodine into rats. As more I^{131} was given the caries experience of the rats increased. Higher doses of the I^{131} also caused more destruction of the thyroid gland, as observed histologically, and atrophy of salivary glands. The authors propose that the salivary glands may serve as intermediates in the thyroid-caries relationship in the rat. Salivary analyses in thyroid deficient children and in adults before and after radioactive therapy offer useful information on this subject.

Cox, Clements, Hilger, and DeGrange (p. 576) found significant enlargement of the submandibular glands of young rats given buffered 0.05 molar acetic, lactic, citric, tartaric or phosphoric acid as their sole drinking fluid for approximately one month. Oxalic acid, at the same concentration, did not result in enlarged salivary glands. While the enlargement with use of acid solutions suggest stimulation as a cause of a compensatory hyperplasia, it does not explain why oxalic acid does not produce such hyperplasia.

Eichel and Swanson (p. 581) established the levels of. total proteins and oxidative enzymes on oral and extraoral tissues from rabbits. The total protein of the liver and submaxillary gland was high, while tongue mucosa, brain cortex, gingiva, and pulp protein was lower. The submaxillary gland also showed relatively high succinic dehydrogenase, DPN cytochrome with reductase and cytochrome oxidase activities with the pulp and gingiva relatively low. The gingiva,

pulp, and submaxillary gland had low catalase activity compared with liver but higher than brain cortex.

Bélanger (p. 595), from a study of rat enamel by autoradiography and microincineration, observed a difference in the process of mineralization in incisors and molars. Although these two teeth grow differently (continuously and limited growth) such a finding is unexpected and can explain "differences" in studies made of teeth which form differently but which might have been expected to mineralize by the same method.

There has been much speculation on the possible harmful effects of airbrasive powder on the patient and dentist. Kerr, Ramfjord, and Ramfjord (p. 602) injected fresh airbrasive powder, used airbrasive powder, powdered enamel, and powdered dentin into the peritoneal cavities of guinea pigs. Mild proliferative reactions were observed in regions containing the new or used airbrasive powder. Enamel and dentin powders also stimulated tissue responses. The authors advise against continued inhalation of airbrasive powder, or powdered enamel, and dentin. While these results are suggestive, their studies on inhalation should be made available before final conclusions are drawn.

Since prolonged local anesthetic effect is undesirable after completion of any operation for which it is used, Luduena (p. 613) investigated the possible effects of various drugs in terminating anestnesia. In guinea pigs, piperoxan, which tends to reduce the vasoconstriction, was most effective of the agents studied. Piperozan, adenosine, and hyaluronidase was the most effective mixed solution reducing the duration of intradermal anesthesia by 10 to 14 per cent. It is possible that the anesthetic effect may be "turned on and off" at will by the use of such agents.

Charbeneau and Peyton (p. 623) describe the application of the shadowed celloidin replica method to sections of teeth containing restorations. Their illustrations of the replicas from the surfaces of sections cut from various types of teeth containing restorations suggest that the method may supply useful information. Especially, the traumatic effects of cavity preparation appear to be available for study by shadowed celloidin replicas.

Mumford (p. 632) has investigated the recurring problem of electromotive forces from dissimilar metals in the mouth. The pain in teeth related to the EMF's is considered and a working hypothesis is proposed. The cessation of pain when the electrodes are in constant contact appears to result from fall in EMF but it is possible that the low EMF, while not a direct cause of pain, may produce pulp damage and thereby, indirectly, pain. Such an hypothesis would be strengthened if pulp damage in teeth under such conditions could be demonstrated experimentally in comparison with controls.

Benkow (p. 641) has devised a simple appliance for establishing radiographic tooth measurements. It should be of value to the practitioner for procedures in which root lengths is important.

Moss and Applebaum (p. 644) present a differential growth analysis for shark, rat, and human teeth. The crown form showed changes in size alone, and simultaneous changes in size and shape. The growth pattern of teeth does not seem to be form or species specific.

The activity of our Japanese Division is indicated by the Proceedings on page 652. The Division met under the chairmanship of Professor Onish with 50 in attendance.

H. B. G. R.

EFFECTS ON MONKEYS OF INTRODUCTION OF HEMOLYTIC STREPTOCOCCI INTO ROOT CANALS

DONALD R. KENNEDY, D.D.S.,* TOM R. HAMILTON, M.D.,** AND JEROME T. SYVERTON, M.D.

The Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.

THE etiology of rheumatic fever has eluded definition for many years; it probably reflects the interaction of heredity, sensitization by previous contact with organisms, and concurrent chronic infection. The role of focal infection is not established although it is known that active rheumatic fever commonly is preceded by an infection with Group A hemolytic streptococci.[1, 2] The isolation of streptococci from a large proportion of of periapical cultures taken at necropsy,[3] and the relative frequency of periapical dental disease, suggest that the latter condition may be considered in the pathogenesis of rheumatic fever.

This paper reports observations made on monkeys in which hemolytic streptococci were sealed aseptically within the root canals of the teeth. The experiments were designed to learn (a) whether these organisms on insertion into the interior of the tooth were productive of root-end infections or systemic effects, (b) whether organisms so inserted or products therefrom were transported to the heart, and (c) whether consequent demonstrable changes representative of the rheumatic type of disease occurred in the heart.

MATERIALS AND METHODS

Experimental animals consisted of 12 monkeys *(Macacus mulatta)* from 39 to 50 months of age.

Organisms inoculated were propagated from strains of *Streptococcus pyogenes hemolyticus,* Groups A and C smooth phase, and Group A matt virulent phase. Four-hour cultures in Todd-Hewitt broth were centrifuged and the supernate removed; a 50 per cent suspension of the centrifugate was prepared in Falba.† The Falba was employed as a vehicle to retain organisms at a desired site with liberation at a slow steady rate.

Aided by a grant from The Division of Research Grants and Fellowships of The National Institutes of Health, Public Health Service, and by a contract between The University of Minnesota and The Office of Naval Research, Department of the Navy (N6 onr 246-T.O. 8).

The material in this paper represents in part the thesis submitted by Donald R. Kennedy in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

Received for publication June 7, 1956.

*Present address: Division of Oral Surgery, College of Dentistry, University of California Medical Center, San Francisco, Calif.

**Present address: Department of Microbiology, University of Kansas Medical Center, Kansas City, Kans.

†Falba is an absorption base of essential oils, aromatic chemicals, and natural and synthetic flower oils. The preparation was obtained from Pfaltz and Bauer, Inc., Empire State Building, New York.

Preparation of monkeys was carried out with the animals under Pentothal sodium anesthesia. Using a routine aseptic technic, selected teeth were isolated with a rubber dam. The area of operation was prepared in the usual manner and the pulp chamber of the tooth entered with a bur. After removal of the pulp the root canal was reamed and filed; instruments were carried beyond the end of the tooth to traumatize the periapical area. The bacteria-Falba suspension was introduced into the root canal of test animals with an absorbent paper point. The procedure was repeated 5 times with fresh inoculum; after the final inoculation, the paper point was covered with a layer of cotton and the tooth sealed with silver amalgam. The same procedure was employed for control animals except that the paper point was sealed in the canal without introduction of organisms ("mock operation").

Observations on animals were made weekly: rectal temperatures, Westergren[4] erythrocyte sedimentation rates, complete blood counts, results of blood cultures, antistreptolysin O titers, and dental roentgenograms were recorded. The Massel modification of the Swift and Todd method[5] was employed for titration of antistreptolysin O levels in serum from blood taken from the popliteal vein. Sera were frozen and stored at 4° C. in stoppered glass tubes until titrated. Blood specimens, 0.5 to 3.0 ml., were taken before operation and at 5, 10, 15, and 30 minute intervals after operations for culture in Todd-Hewitt broth at 37° C. Cultures were examined microscopically daily for 4 days and on the seventh postoperative day. Subcultures were made, on days 1 to 4 and on day 7, to 3 per cent rabbit blood agar plates incubated at 37° C. Plates were incubated for 72 hours before being discarded as negative. Suspected streptococcal colonies were isolated in pure culture and the organisms identified morphologically. At autopsy, root canal cultures were obtained after aseptic removal of the sealing amalgam by immersion of the paper point in Todd-Hewitt broth. The cultures were treated further as outlined for blood specimens. Also, at autopsy, the teeth and surrounding structures, and the hearts and greater vessels were removed and sections made.

RESULTS

Experimental schedules for the introduction of organisms into the teeth and for mock operations were followed as shown in Tables I and II. The 12 experimental monkeys, divided into 5 groups with respect to age and sex, were caged separately with individual supplies of food and water. Observations in Experiment 1 were begun 2 weeks before the first operation, those in Experiment 2 on the day of the first operation. Succeeding observations were made immediately before the following operations. The animals of Experiment 1 were killed at 2 weeks, those of Experiment 2, 4 or 7 weeks after the final operation.

Rectal temperatures, sedimentation rates, and blood counts exhibited little or no definite pattern, as can be seen from the illustrative results in Table III. For instance, XM#8 showed a decrease in erythrocytes, but so did CM#4;

TABLE I
SCHEDULE OF TREATMENT OF MACACUS MULATTA MONKEYS, EXPERIMENT 1

GROUP	MONKEY	SEX	AGE* (MONTHS)	EXPERIMENTAL TEETH INOCULATED	STREPTOCOCCI INOCULATED LANCEFIELD GROUP AND TYPE		
					Strain		
I	XM#1†	Male	48	1|	592SD	A	17
	CM#2	Male	46	2|	Mock operation		
II	XM#3	Female	39	|1	C-17	C	
				1|	CO	A	19
	CM#4	Female	42	|1	Mock operation		
				1|	Mock operation		
III	XM#5	Male	46	|1	C-17	C	
				1|	CO	A	19
				|2	592SD	A	17
	CM#6	Male	43	|1	Mock operation		
				1|	Mock operation		
				|2	Mock operation		
IV	XM#7	Female	50	|1	C-17	C	
				1|	CO	A	19
				|2	592SD	A	17
				2|	C-17	C	
				|3	CO	A	19
				3|	592SD	A	17
	XM#8	Female	50	|1	C-17	C	
				1|	C-17	C	
				|2	CO	A	19
				2|	CO	A	19
				|3	592SD	A	17
				3|	592SD	A	17
	CM#9	Male	50	|1	Mock operation		
				1|	Mock operation		
				|2	Mock operation		
				2|	Mock operation		
				|3	Mock operation		
				3|	Mock operation		

*Age estimated from Hartman age classification according to erupted permanent dentition (Hartman, C. G., and Straus, W. L.: *Anatomy of the Rhesus Monkey*, Baltimore, 1933, Williams & Wilkins Co., p. 87).

†XM denotes an experimental monkey; CM denotes a control monkey.

although XM#1 and XM#7 showed increased eosinophil counts, CM#4 did likewise. In Experiment 2, XM#11 and XM#12 did exhibit, for the monkey, an increase in sedimentation rate the first week after the last inoculation, to reach, respectively, 7.5 and 10.5 mm. in 60 minutes, but the rates returned to normal the following week, and only XM#12 was found to have had a Group A streptococcal bacteremia.

TABLE II
SCHEDULE OF TREATMENT OF MACACUS MULATTA MONKEYS, EXPERIMENT 2

GROUP	MONKEY	SEX	AGE (MONTHS)	EXPERIMENTAL TEETH INOCULATED	STREPTOCOCCI INOCULATED LANCEFIELD GROUP AND TYPE		
V	XM#10	Female	50	$\frac{1}{\|}$	A	19	2848F
				$\frac{\|1}{}$	A	17	592SD
				$\frac{2}{\|}$	A	3	V-1729
				$\frac{\|2}{}$	A	4	T-4
	XM#11	Female	50	$\frac{1}{\|}$	A	19	2848F
				$\frac{\|1}{}$	A	17	592SD
				$\frac{2}{\|}$	A	3	V-1729
				$\frac{\|2}{}$	A	4	T-4
	XM#12	Female	50	$\frac{1}{\|}$	A	19	2848F
				$\frac{\|1}{}$	A	17	592SD
				$\frac{2}{\|}$	A	3	V-1729
				$\frac{\|2}{}$	A	4	T-4
				$\frac{3}{\|}$	A	17	592SD

Blood cultures in Experiment 1 were negative. In Experiment 2, XM#10 and XM#12 demonstrated transient bacteremias produced by the inoculated organism; in the second monkey the blood invasion persisted after the operation for 15 minutes.

Root canal cultures yielded viable streptococci in the case of 5 of the inoculated monkeys. Recovery of the organism from one animal (XM#12) after 9 weeks is in accord with the view that Lancefield Group A hemolytic streptococci are only occasionally pathogenic for monkeys. Table IV illustrates the types of organisms isolated in root canal cultures.

X-ray examinations indicated that the root-ends of only 2 teeth were affected pathologically; on the remaining teeth no radiolucency could be determined.

Antistreptolysin O titers of blood obtained initially from the 12 monkeys ranged from 57 to 317 units. With 4 inoculated animals (XM#5, 7, 11, 12) greater than twofold elevations of titer were observed; of the 4 animals, XM#5 exhibited a twofold rise on the day of operation but prior to operation. Monkeys receiving 4 or more inoculations revealed the highest titers: 833 (XM#11) and 1,200 (XM#12) units. The latter monkey, which possessed the most striking cardiac lesions and evidenced a transient bacteremia after inoculation, maintained the high titer for several weeks (Fig. 1). There were no significant elevations in titer follwoing mock operations.

Histopathologic examination of the periapical areas showed little difference between inoculated and uninoculated animals. The pathologic evidence (osteoclastic activity, inflammation, infiltration of lymphocytes and polymorphonuclear cells) was variable and without pattern in all of the animals.

TABLE III
RECTAL TEMPERATURES, DIFFERENTIAL BLOOD COUNTS, AND ERYTHROCYTE SEDIMENTATION RATES DETERMINED FOR MONKEYS OF GROUP IV

	WEEKS BEFORE OPERATION		OPERATION NUMBER						WEEKS AFTER OPERATION	
	2	1	1	2	3	4	5	6	1	2
XM#7										
Rectal temp. ° F.—100	1.6	1.6	2.4	3.0	4.0	2.7	3.4	3.0	3.2	1.8
RBC × 1,000,000	5.0	5.0	5.2	4.2	4.5	4.2	4.1	4.7	4.7	4.2
WBC × 1,000	20.0	25.7	15.4	17.3	14.8	9.1	12.6	17.2	9.8	14.3
Polymorphonuclear cells %	21.9	55.33	19.10	28.50	30.20	26.60	23.3		34.38	20.7
Leukocytes %	67.03	42.29	79.1	68.96	68.9	72.4	76.7		64.7	74.7
Eosinophils %	0.0	2.0	0.9	1.5	0.4	0.75	0.0		0.46	3.45
Basophils %	8.78	0.0	0.0	0.0	0.2	0.0	0.0		0.0	0.15
Monocytes %	2.1	0.38	0.9	0.6	0.2	0.25	0.0		0.46	0.0
Sed. Rate/Min.										
20	0.0	0.0	0.0	0.0	0.25	0.25	0.25	0.0	0.5	0.25
40	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25
60	0.5	0.5	0.5	0.5	0.5	0.25	0.25	1.5	1.0	0.5
80	1.0	1.0	1.0	1.0	0.5	0.5	0.5	2.0	1.5	1.0
XM#8										
Rectal temp. ° F.—100	2.5	1.6		4.4	3.4	4.0	3.0	3.8	2.0	2.5
RBC × 1,000,000	5.4	5.0	5.0	6.7	4.2	3.4	4.2	4.1	5.1	4.9
WBC × 1,000	13.0	25.7	16.5	11.1	8.2	7.7	7.0	6.7	12.1	7.6
Polymorphonuclear cells %	51.50	55.33	27.80	35.10	45.2	64.0		50.0	37.0	79.5
Leukocytes %	48.0	42.29	71.7	64.20	43.8	44.0		49.0	62.4	20.0
Eosinophils %	0.5	2.0	0.5	0.0	0.5	2.0		0.5	0.0	0.5
Basophils %	0.0	0.0	0.0	0.7	0.5	0.0		0.5	0.6	0.0
Monocytes %	0.5	1.0	1.0	0.0	0.0	0.0		0.0	0.0	0.0
Sed Rate/Min.										
20	0.0	0.0	0.0	0.0		0.0	0.25	0.0	0.0	0.0
40	0.25	0.25	0.25	0.25		0.0	0.25	0.25	0.0	0.25
60	0.5	0.5	0.5	0.5		0.25	0.25	0.25	0.25	0.5
80	1.0	0.5	0.5	1.0		0.25	0.25	0.5	0.25	0.5
CM#9										
Rectal temp. ° F.—100		2.7	3.2	3.6	3.2	4.0	4.2	3.0	2.0	2.0
RBC × 1,000,000	5.5	5.8	5.3	5.8	5.7	5.1	5.8	4.9	5.3	5.5
WBC × 1,000	17.3	13.8	13.0	10.7	11.2	9.3	9.5	14.3	11.2	12.3
Polymorphonuclear cells %	40.0	20.4	42.7	28.1	27.1	39.0		62.27	16.5	30.7
Leukocytes %	57.2	78.26	55.6	68.9	66.3	60.0		36.3	88.1	65.3
Eosinophils %	1.8	0.43	0.8	1.2	4.5	1.0		1.42	1.4	2.6
Basophils %	1.12	0.0	1.6	1.2	0.9	0.0		1.0	0.0	0.7
Monocytes %	0.0	0.86	0.8	0.6	0.9	0.0		0.0	0.0	0.7
Sed. Rate./Min.										
20	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
40	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.5	0.25	0.25
60	0.5	0.5	0.5	0.5	0.5	0.75	0.5	1.0	0.5	0.25
80	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0	0.5	0.25

Histologic observation of the hearts of the monkeys, made on 7 sections per heart, revealed evidence of inflammation in at least one area of the heart or great vessels of each monkey. Myocarditis was present in 11 of the animals: XM#7, the animal without histopathologic change in the cardiac musculature, had paradoxically received the largest number (6) of streptococcal inoculations. In this animal, a 2+ pulmonary arteritis was associated

TABLE IV
RESULTS OF BACTERIOLOGIC EXAMINATION OF ROOT CANALS OF MONKEYS OF GROUP IV

MONKEY	TOOTH	INOCULUM	RESULTS OF CULTURE AT AUTOPSY
XM#7	$\frac{\|1}{}$	C-17	Contaminants
	$\frac{1\|}{}$	M-27	Contaminants
	$\frac{\|2}{}$	A-17	Contaminants
	$\frac{2\|}{}$	C-17	Contaminants
	$\frac{\|3}{}$	M-27	Hemolytic staphylococcus
	$\frac{3\|}{}$	A-17	Contaminants
XM#8	$\frac{\|1}{}$	C-17	Group A streptococcus
	$\frac{1\|}{}$	C-17	Group C streptococcus
	$\frac{\|2}{}$	M-27	Contaminants
	$\frac{2\|}{}$	M-27	Contaminants
	$\frac{\|3}{}$	A-17	Group C streptococcus
	$\frac{3\|}{}$	A-17	Group A streptococcus
XM#9	$\frac{\|1}{}$	Mock operation	Diplococcal organisms
	$\frac{1\|}{}$	Mock operation	Diplococcal organisms
	$\frac{\|2}{}$	Mock operation	Diplococcal organisms
	$\frac{2\|}{}$	Mock operation	Sterile
	$\frac{\|3}{}$	Mock operation	Sterile
	$\frac{3\|}{}$	Mock operation	Diplococcal organisms

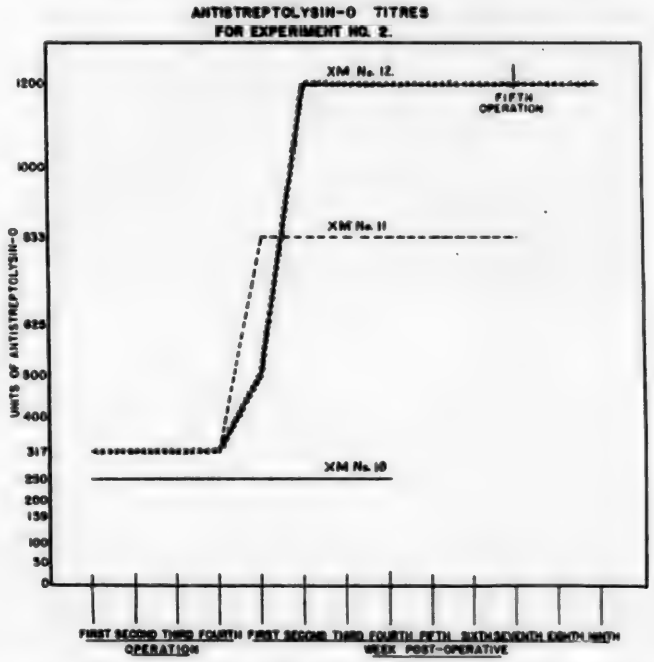


Fig. 1.—Antistreptolysin O titers of monkey after inoculation of experimental teeth with streptococcal cultures.

with adjacent periarterial involvement and pericarditis in corresponding areas of the subepicardial region. Monkey XM#8, treated similarly, showed only small 1+ foci of myocardial lymphocytic infiltrations. Myocarditis of 2+ intensity was observed after mock operations in 3 of 4 monkeys. Although lymphocytic accumulations commonly characterized myocardial changes, in the 3 animals with the most severe lesions some zones were infiltrated with polymorphonuclear leukocytes even following mock operations. Necrosis was noted in only 2 hearts.

Significant (1+) changes in the valvular endocardium appeared in only 2 monkeys, XM#10 and XM#12. The latter, examined 2 weeks after the fifth inoculation of virulent streptococci (Group A types 3, 17, 19, 4, and 17 given in sequence with an interval of 5 weeks between the last 2 inoculations) exhibited the most striking lesions. The former monkey (XM#10) received

Fig. 2.—Orange brown deposit observed on teeth of some experimental animals.

only 4 duplicate inoculations. Monkey XM#12 also showed the most pronounced inflammatory reaction (3+), with palisading of cells in the subendothelial zone coursing along the media in the pulmonary artery. The presence of basophilic tissue cells in the zone of cellular infiltration and proliferation gave a "rheumatic-like" appearance to the lesions. The endothelial cells lining the artery and adjacent small vessels in subepicardial fat were swollen and in many instances multinucleated. Swollen proliferating cells appeared also in the mesothelial lining of the visceral pericardium, which overlies the pulmonary artery. Both XM#10 and XM#12, from which blood cultures of the same streptococcal type inoculated were isolated, had myocarditis of 2+ severity.

Perivascular reaction in this dental series was limited virtually to that associated with the unusual type of inflammatory involvement of the pulmonary artery. The single exception was 1+ lymphocytic accumulation in a monkey (XM#5) inoculated once each with Groups A and C streptococci.

Visual observation of the teeth of the monkeys revealed an orange-brown deposit around the necks of the teeth of some of the inoculated monkeys (see Fig. 2).

DISCUSSION

This study was conducted to investigate the possible role of streptococcal tooth infection in the etiology of the rheumatic type of cardiovascular disease. It was found that a transient bacteremia could be produced by inoculation of the tooth root canal with streptococci, and that at terminal examination the same organism could be recovered from the root canal immediately post mortem. In the majority of instances, experimental lesions on root-ends were not demonstrated radiographically. Few significant systemic effects of inoculation, such as fever, elevated blood counts, or increased erythrocyte sedimentation rates, were observed. Greater than twofold elevations of antistreptolysin O titer, absent in controls, occurred in 3 inoculated monkeys. Histopathologic examinations showed lesions on the root-ends of the teeth but, in the case of the heart, "naturally" occurring changes in the myocardium and pulmonary artery[6] made evaluation of the response to inoculation difficult. It should be noted that the control animals could not be considered normal since they were subjected to the stress of mock operations. In 2 monkeys mild vascular changes, otherwise absent, followed multiple inoculations of different virulent streptococci; these animals also responded serologically. The host response was independent of the sequence of streptococcal types given in multiple injections.

Numerous theories of the etiology of rheumatic fever have been offered: anaphylactic and allergic phenomena,[7-11] auto-antibody production,[12] and adrenal cortical abnormalities all have been postulated as basic causes.[13] It is commonly conceded[14-17] that streptococcal infection is related to the disease, although no one type of organism has been successfully incriminated.[18, 19] Definition of the relation has been hindered by the failure of experimental animals to respond with the complete pathologic picture so well delineated in human beings. Similar but not identical syndromes have been induced in animals by various means. Acute rheumatic-like heart lesions have been produced in mice by parenteral injections of such foreign proteins as egg white.[20] Murphy and Swift[21] observed cardiac lesions in rabbits with multiple skin injections of several serologic types of Group A streptococci; Loewe and Lenke[22] were able to produce, in rabbits, cardiac lesions resembling the Aschoff bodies commonly accepted as pathognomonic for rheumatic fever.[23] The cardiac lesions were accompanied by a pancarditis which the investigators considered might be the counterpart of human rheumatic pancarditis. With normal and hypersensitized rabbits and monkeys, Hamilton and Syverton[6]

found that cardiovascular inflammatory changes followed intravenous injections of streptococci alone or combined with foreign proteins. The more rheumatic-like lesions were observed in rabbits; in monkeys, arteritis and periarteritis were more common. In animal experimentation, then, the relation of observed tissue changes to those of the human disease is a primary consideration.

A rational basis for the present study is provided by these facts.

1. Experimental root-end infections can be produced in monkeys. Genvert, Miller, and Burn[24] produced experimental lesions of the teeth in monkeys by exposure of the pulp canals and by direct introduction of known bacteria. These induced lesions were considered indistinguishable from those observed clinically in man.

2. Local infection originating in the teeth has been thought to play a significant role in the pathogenesis of chronic disease.[25-28] Welch, Murcock, and Ferguson[29] injected streptococci into the teeth of rabbits previously sensitized and subjected to low-grade infection, with resultant production of a subacute bacterial endocarditis.

3. Bacteremias may be associated with local infection and can originate from the teeth. It is commonly accepted, as Reichel[30] has stated, that a bacteremia does not usually persist in the absence of focal infection. Transient bacteremias have been produced experimentally by injection of streptococci into the tooth root canals of rabbits,[29] and by the introduction of nonpathogenic bacteria into the gingival crevice prior to tooth extraction,[31] showing that bacteremia can be of periodontal origin. Transient bacteremias have been associated also clinically with extraction or disturbance of the teeth.[32-35]

4. The blood picture in rheumatic fever may be useful diagnostically. In mild cases the leukocyte count may be slightly elevated,[2] and it has been claimed that repeated observations of eosinophil response may provide an index of rheumatic infection.[36] With exacerbation, a moderate or severe anemia may develop.[37] The sedimentation rate usually increases during the active stages of rheumatic fever and may remain elevated after the disease apparently has subsided.[2] A systemic effect of rheumatic disease may be an increase in the sedimentation rate.

The results of the study, concerning the production of root-end lesions and transient bacteremias of dental origin, agree with those of other investigators. No evidence of the systemic effects which might be expected to accompany transport of the inoculated organisms to the heart was obtained. In none of the test animals in these dental experiments was there evidence of the coronary arteritis such as observed in other studies of experimental cardiovascular disease made by Hamilton and Syverton.[38] Failure to demonstrate heart changes typical of rheumatic disease, although suggestive in one monkey, may have resulted from: (a) inability of the animals to respond in human fashion, as has been postulated by Murphy and Swift,[21] and Loewe and

Lenke[22]; (b) insusceptibility of these animals to the microorganisms pathogenic in man; or (c) absence of an unknown potentiating factor essential to production of the rheumatic condition.

The deposit observed on the teeth of some of the experimental monkeys is interesting in view of Entine's[39] suspicion that similar deposits may be symptomatic in some cases of rheumatic fever.

SUMMARY

Bacteriologic and histopathologic studies have been made on the effects of inoculation of streptococci into the tooth root canals of 8 *Macacus mulatta* monkeys. It was shown that transient bacteremias could be produced by such inoculation, and that the inoculated organisms were recoverable from tooth root canals at terminal examination. Experimental root-end lesions were revealed by histopathologic, but rarely by radiographic, examination. Signs of systemic effects in the 8 inoculated monkeys were limited to significant responses in antistreptolysin O titer in 3 animals, and minor increases in erythrocyte sedimentation rate in 2 cases. The experimental treatment did not result in cardiac changes typical of rheumatic disease.

REFERENCES

1. Holbrook, W. P.: Rheumatic Fever, *Hygeia* **25**: 344, 1947.
2. Dowling, H. F.: The Acute Bacterial Diseases, Philadelphia and London, 1948, W. B. Saunders Company, p. 163.
3. Burkett, L. W.: Studies of the Apices of Teeth, *Yale J. Biol. & Med.* **9**: 347, 1937.
4. Todd, J. C., Sanford, A. H., and Wells, B. B.: Clinical Diagnosis by Laboratory Methods, ed. 12, Philadelphia and London, 1953, W. B. Saunders Company.
5. Massel, B. F., Dow, J. W., and Jones, T. D.: Orally Administered Penicillin in Patients With Rheumatic Fever, *J. A. M. A.* **138**: 1030, 1948.
6. Hamilton, T. R., and Syverton, J. T.: Experimental Studies in Cardiovascular Disease, Rheumatic Type, *Bull. Univ. Minnesota Hosp. & Med. Found.* **21**: 173, 1950.
7. Bruun, E.: Allergische Arthritis and Myokarditis, *Virchows Arch. f. path. Anat.* **303**: 524, 1939.
8. Altman, R., and Gersner, L.: Ueber die Bedeutung der Tonsillen, bzw. des peritonsillaren Gewebes für das Zustandekommen hypergisch entzundlicher Gewebsvaranderungen auf tuberkuloser Basis, *Virchows Arch. f. path. Anat.* **296**: 480, 1935.
9. Freiberg, J. A.: Allergy as a Factor in the Production of Proliferative Arthritis, *Arch. Surg.* **18**: 645, 1929.
10. Baker, B. M., Thomas, C. B., and Penick, R. M.: Experimental Carditis. Changes in the Myocardium and Pericardium of Rabbits Sensitized to Streptococci, *J. Clin. Invest.* **14**: 465, 1935.
11. Atwater, R. M.: Studies in the Epidemiology of Acute Rheumatic Fever and Related Diseases in the United States, Based on Mortality Statistics, *Am. J. Hyg.* **7**: 343, 1927.
12. Cavelti, P. A.: Studies on the Pathogenesis of Rheumatic Fever, *Arch. Path.* **44**: 1, 1947.
13. Selye, H., and Pentz, E. I.: Pathogenetical Correlations Between Periarteritis Nodosa, Renal Hypertension and Rheumatic Lesions, *Canad. Med. A. J.* **49**: 264, 1943.
14. Coburn, A. F.: The Factor of Infection in the Rheumatic State, Baltimore, 1931, Williams & Wilkins Company, p. 47.
15. Griffith, G. C.: Rheumatic Fever, Its Recognition and Treatment, *J. A. M. A.* **133**: 974, 1947.
16. Holbrook, W. P.: The Army Air Force Rheumatic Fever Control Program, *J. A. M. A.* **126**: 84, 1944.
17. Swift, H. F.: The Relationship of Streptococcal Infections to Rheumatic Fever, *Am. J. Med.* **2**: 168, 1947.
18. Van Ravenswaay, A. C.: The Geographic Distribution of Hemolytic Streptococci, Relationship to the Incidence of Rheumatic Fever, *J. A. M. A.* **126**: 486, 1944.

19. Watson, R. F., Rothbard, S., and Swift, H. F.: The Relationship of Post-scarlatinal Arthritis and Carditis to Rheumatic Fever, *J. A. M. A.* **128**: 1145, 1945.
20. Moore, F. J., Ridge, G. J., Huntington, R. W., Hall, E. M., Griffith, G. C., and Knowles, R. G.: Production of Acute Rheumatic-like Heart Lesions in Mice, *Proc. Soc. Exper. Biol. & Med.* **65**: 102, 1941.
21. Murphy, G. E., and Swift, H. F.: Induction of Cardiac Lesions Closely Resembling Those of Rheumatic Fever, in Rabbits Following Repeated Skin Infections With Group A Streptococci, *J. Exper. Med.* **89**: 687, 1949.
22. Loewe, L., and Lenke, S. E.: Cardiac Lesions Resembling Aschoff Bodies in Rabbits, *J. Exper. Med.* **71**: 89, 1940.
23. Laipply, T. C., and O'Neill, J. B.: Vascular Lesions in Rheumatic Fever, *Quart. Bull. Northwestern Univ. M. School* **21**: 211, 1947.
24. Genvert, H., Miller, H., and Burn, C. G.: Experimental Production of Apical Lesions of Teeth in Monkeys and Their Relation to Systemic Disease, *Yale J. Biol. & Med.* **13**: 649, 1941.
25. Crowe, H. W.: Oral Sepsis in Relation to Rheumatic Conditions, *Brit. D. J.* **68**: 173, 1940.
26. Cecil, R. L., Nicholls, E. E., and Stainsby, W. J.: The Bacteriology of the Blood and Joints on Chronic Infectious Arthritis, *Arch. Int. Med.* **43**: 571, 1929.
27. Stein, G.: Newer Aspects of the Problem of Focal Infection, *Am. J. Orthodont. & Oral Surg.* **28**: 28, 1942.
28. Thoma, K. H.: Infected Vital Dental Pulp, Important Focus of Systemic Diseases, *J. D. Res.* **9**: 529, 1928.
29. Welch, H., Murcock, T. P., and Ferguson, J. A.: Subacute Bacterial Endocarditis Production in Rabbits With Streptococci That Resembles Diphtheroids, *J. Lab. & Clin. Med.* **21**: 1264, 1936.
30. Reichel, H. A.: The Removal of Bacteria From the Blood Stream: The Experiments Tending to Determine the Rate of Removal of Injected Bacteria in the Blood, *Proc. Staff Meet. Mayo Clin.* **14**: 138, 1939.
31. Burket, L. W., and Burn, G. C.: Bacteremias Following the Dental Extractions: Demonstration of Source of Bacteria by Means of Non-Pathogen (*Serratia marcescens*), *J. D. Res.* **6**: 521, 1937.
32. Hirsh, H. L., Vivina, J. J., Merril, A., and Dowling, H. F.: The Effects of Prophylactically Administered Penicillin on Incidence of Bacteremia Following the Extraction of Teeth, *Arch. Int. Med.* **81**: 868, 1948.
33. Faillo, P. S.: Blood Findings on Twenty Patients Before and After Extraction of Teeth, *J. D. Res.* **21**: 19, 1942.
34. Richard, J. K.: Bacteremia Following Irritations of Foci of Infection, *J. A. M. A.* **99**: 1496, 1932.
35. Murray, M., and Moosnick, F.: The Incidence of Bacteremia in Patients With Dental Disease, *J. Lab. & Clin. Med.* **26**: 801, 1941.
36. Friedman, G., and Holtz, E.: The Behavior of the Eosinophiles in Rheumatic Fever, *J. Lab. & Clin. Med.* **21**: 225, 1935.
37. Hubbard, J. P., and McKee, M. H.: Anemia of Rheumatic Fever, *J. Pediat.* **14**: 66, 1939.
38. Hamilton, T. R., and Syverton, J. T.: Experimental Cardiovascular Disease: The Problem of Natural Occurring Carditis in Monkeys. Read before the North Central Branch of The Society of American Bacteriologists Meeting, Minneapolis, Minnesota, October 13, 1953.
39. Entine, M.: A Survey of Dental Diseases as a Diagnostic Aid in Rheumatic Fever. *J. A. D. A.* **38**: 303, 1949.

PLACENTAL PERMEABILITY TO BACTERIAL TOXINS

HOLMES T. KNIGHTON, D.D.S., SEYMOUR J. KRESHOVER,* D.D.S., M.D., PH.D., AND JAMES A. HANCOCK, JR., M.S.

From the Departments of Microbiology and Dental Research, The Medical College of Virginia, Richmond, Va.

RECOGNIZING the importance of a better understanding of the nature of prenatal influences on the development of dental tissues, certain aspects of congenital disease seem to warrant additional consideration.

Although the ability of certain diseases to be transferred from mother to fetus has long been recognized, the precise mechanism by which this may occur has not been clarified. Such possible mediating factors as a disturbed maternal metabolism and passage of microorganisms or their toxins across an intact or diseased placenta must be considered. However, knowledge to date on the relationship of infectious diseases to pregnancy and the fetus has been gained largely by inference and hypothesis. Klautsch emphasized the conflict of opinions and suggested that although direct transfer of infection is possible under certain conditions, it is far less important than fetal disturbances caused by metabolic changes or pathosis of the endomentrium and placenta.[1] Nevertheless, many clinical reports of congenital infection, both bacterial and viral, have appeared in the literature.[2-5] The preponderance of opinion would suggest that a break in the placental barrier is a prerequisite for transplacental passage of infectious agents.[6]

It is generally assumed that maternal-fetal transfer of bacterial toxins does not occur, although little experimental evidence is available to support this contention. Further difficulty is met by the controversial nature of the few reported studies. As an example, Schmidlechner[7] demonstrated placental crossing of diphtheria toxin following subcutaneous injections of large doses in pregnant guinea pigs, whereas Mouriquand, Leulier, and Sedallian,[8] as well as Nattan-Larrier, Ramon, and Grasset,[9] found no evidence of passage in similar studies on the guinea pig and rabbit. The latter investigators, repeating the same type of study with tetanus toxin, demonstrated placental crossing in the guinea pig but not in the rabbit.

Evaluating the question of transplacental passage of diphtheria toxin in human beings, Robinson and co-workers surveyed 50,000 obstetrical case reports in which there occurred 5 instances of maternal diphtheria.[10] In none of the

Presented at the Thirty-third Meeting of the International Association for Dental Research, March 18-20, 1955, Chicago, Ill. (*J. D. Res.* 34: 703, 1955.)

This study was supported by a grant from the United States Public Health Service.

Received for publication Sept. 20, 1956.

*Present address: National Institute of Dental Research, Bethesda 14, Md.

latter was infection noted in the delivered young. Other reports have been cited in the literature suggesting the untoward effect of maternal bacterial toxemias on the course of pregnancy with particular respect to abortion.[11, 12]

MATERIALS AND METHODS

The present study was designed in order to determine whether certain toxins have the capacity to cross the placental barrier. On the basis of availability and potency, the exotoxins of *Cl. tetani, Cl. oedematiens,* and *C. diphtheriae* were selected for initial study.

Rabbits, guinea pigs, and rats were chosen for this experiment because of the similarity of their placental structure to that of man.[13] Groups of these animals in their second and third trimesters of pregnancy were injected by different routes with varying amounts of toxin (Tables I through IV). Trimesters were arbitrarily established as 7 days for rats, 10 days for rabbits, and 22 days for guinea pigs. Maternal blood samples and fetuses were removed aseptically at post-injection intervals between 15 minutes and 7 hours in order to determine the presence or absence of toxin in the respective tissues. Following a midline abdominal incision, the uterus was clamped just proximal to the most distally positioned fetus in the left horn. The wall was then seared with a heated spatula prior to exposing the amniotic sac, and the fetus carefully extracted so as to avoid contact with maternal tissues. The umbilical cord was initially severed in order to permit independent removal of the placenta. Wound clips were used to close the abdomen until such time as the next fetus was scheduled for examination.

In order to test for the presence of tetanus and oedematiens toxins, the hind legs of mice were injected with 0.01 to 0.2 ml. of maternal blood and 0.1 to 0.4 ml. of fetal blood. Diphtheria toxin was tested for in guinea pigs by subcutaneous injection of 0.2 ml. of maternal blood and 0.2 to 0.5 ml. of fetal blood. All test animals were then observed for 4 to 5 days and recordings made relative to lethal or reactionary effects. Inasmuch as adequate blood samples could be obtained only from third trimester fetuses, the young of rabbits and guinea pigs, sacrificed at earlier periods of gestation, were macerated in order to prepare material for injection into test animals.

Further experimental procedures were designed in order to determine whether placental permeability to toxins might be enhanced by a histamine-releaser (dextran), or by the spreading factor of hyaluronidase. Accordingly, rats in their third trimester of pregnancy were injected with varying doses of these agents, as well as with 10,000 to 20,000 (mouse) minimum lethal dose (MLD) of tetanus toxin. A schedule of routes of inoculation and time of testing maternal blood and fetuses for toxin is recorded in Table IV.

Prior to initiating the outlined experimental procedures, it seemed advisable to obtain some information regarding the minimum amount of toxin that could be detected in fetal tissue. For this purpose, tetanus toxin was diluted in normal saline, and 0.1 ml. lots each containing 1 to 500 MLD, were injected directly

into third trimester rabbit fetuses. To provide a control measure against nonspecific results, representative amounts of all blood and macerated fetal specimens were injected into antitoxin protected test animals.

FINDINGS

Test for the detection of tetanus toxin in fetal blood and macerated tissues were uniformly negative (Table I). On the other hand, maternal blood specimens were found to contain this toxin in varying amounts per milliliter. In that group of 5 rabbits receiving single intravenous injections during their second and third trimesters of pregnancy, the maternal blood demonstrated at least 10 MLD per milliliter during the first 30 minutes of post-inoculation testing, and at least 10 reactionary doses per milliliter through the entire 120-minute observation period. Another group of 4 second trimesters rabbits injected intravenously with 75,000 MLD, followed 2 hours later by a duplicate inoculation, showed at least 5 MLD of toxin per milliliter in maternal blood samples obtained during the 2-hour testing period. In a third series of 4 rabbits that were injected subcutaneously with 200,000 MLD, followed by 7 intravenous injections of 20,000 MLD at hourly intervals, there was at least 100 MLD per milliliter in the maternal blood samples obtained 15 minutes after the last injection.

TABLE I
SCHEDULE OF EXPERIMENTS WITH TETANUS TOXIN

DOSAGE (MOUSE) MLD	ANIMALS	TIME OF INOCULATION (TRIMESTER OF PREGNANCY)	TIME OF TESTING MATERNAL BLOOD AND FETUSES FOR TOXIN (MINUTES FOLLOWING LAST INOCULATION)	DEMONSTRATION OF TOXIN	
				MATERNAL	FETAL
50,000 to 75,000 intravenously	2 rabbits	2	15, 30, 60, 120	Positive	Negative
	2 rabbits	3			
75,000 intravenously plus 75,000 intravenously one hour later	2 rabbits	2			
	2 rabbits	3	15, 30, 60, 120	Positive	Negative
200,000 subcutaneously plus 20,000 intravenously every hour for seven doses	3 rabbits	3	15	Positive	Negative
20,000 intracardially	1 guinea pig	3	30, 60	Positive	Negative
200,000 subcutaneously plus 40,000 intracardially	2 guinea pigs	2	420	Positive	Negative
	3 guinea pigs	3			

Although an additional group of 6 guinea pigs that received tetanus toxin likewise demonstrated a consistently positive blood picture, the MLD per milliliter was uniformly higher. This is shown by the 100 MLD per milliliter level which followed a single intracardial injection of 20,000 MLD. Also noted in the guinea pig series was a prolonged toxemia as illustrated by the presence of at

least 10 MLD per milliliter for a 7-hour period following simultaneous intracardial and subcutaneous injections of 40,000 and 200,000 MLD doses, respectively.

Evaluating the effectiveness of the placenta in blocking the passage of diphtheria toxin (Table II), samples of fetal blood and macerated tissue were uniformly negative for the presence of toxin. In contrast, the maternal blood of inoculated rabbits and guinea pigs showed consistent evidence of toxemia. For example, 3 pregnant rabbits receiving either a single intravenous injection of 45,000 MLD or twice this amount in divided doses, showed enough toxin in 0.2 ml. of their blood to cause death in unprotected guinea pigs throughout a 3-hour sampling period. Similar findings over a 7-hour testing period were noted in 3 pregnant guinea pigs injected subcutaneously with 10,000 MLD and intracardially with 2,000 MLD.

TABLE II
SCHEDULE OF EXPERIMENTS WITH DIPHTHERIA TOXIN

DOSAGE (GUINEA PIG) MLD	ANIMALS	TIME OF INOCULATION (TRIMESTER OF PREGNANCY)	TIME OF TESTING MATERNAL BLOOD AND FETUSES FOR TOXIN (MINUTES FOLLOWING LAST INOCULATION)	DEMONSTRATION OF TOXIN	
				MATERNAL	FETAL
45,000 intravenously	1 rabbit	3	15, 30, 60, 120	Positive	Negative
45,000 intravenously plus 45,000 intravenously one hour later	2 rabbits	3	120, 180	Positive	Negative
10,000 subcutaneously plus 2,000 intracardially	3 guinea pigs	3	420	Positive	Negative

In experiments with oedematiens toxin (Table III), results were relatively similar to those previously described. The 8,000 and 16,000 (mouse) MLD of toxin injected into 3 rabbits during the third trimester of pregnancy was not evident in fetal blood. On the other hand, at least 10 MLD per milliliter of toxin was present in maternal blood throughout the period of 120 minutes following intravenous injection.

TABLE III
SCHEDULE OF EXPERIMENTS WITH OEDEMATIENS TOXIN

DOSAGE (MOUSE) MLD	ANIMALS	TIME OF INOCULATION (TRIMESTER OF PREGNANCY)	TIME OF TESTING MATERNAL BLOOD AND FETUSES FOR TOXIN (MINUTES FOLLOWING LAST INOCULATION)	DEMONSTRATION OF TOXIN	
				MATERNAL	FETAL
8,000 intravenously	1 rabbit	3	15, 30, 60, 120	Positive	Negative
16,000 intravenously	2 rabbits	3	120	Positive	Negative

A schedule of experiments related to the influence of dextran and hyaluronidase on placental passage of tetanus toxin is listed in Table IV. Results were uniformly negative for presence of tetanus toxin in fetal blood or tissues. In contrast, at least 10 MLD per milliliter of toxin was present in the maternal blood of each of the 7 rats throughout the 4- to 5-hour periods used in this study.

TABLE IV
SCHEDULE OF EXPERIMENTS WITH TETANUS TOXIN IN THE PRESENCE OF HYALURONIDASE AND DEXTRAN

AGENTS INOCULATED	ANIMALS	TIME OF INOCULATION (TRIMESTER OF PREGNANCY)	TIME OF TESTING MATERNAL BLOOD AND FETUSES FOR TOXIN (MINUTES FOLLOWING LAST INOCULATION)	DEMONSTRATION OF TOXIN	
				MATERNAL	FETAL
10,000 (mouse) MLD tetanus toxin intracardially and 1,000 TRU hyaluronidase intracardially	1 rat	3	240	Positive	Negative
20,000 (mouse) MLD tetanus toxin intracardially and 300 TRU hyaluronidase intraplacentally	2 rats	3	300	Positive	Negative
10,000 (mouse) MLD tetanus toxin intracardially and 0.25 to 0.5 ml. of 6% dextran intraperitoneally	4 rats	3	300	Positive	Negative

Recognizing that the failure to demonstrate placental passage of toxin is accurate only in so far as testing methods can assure detection of stated amounts, it is important to note that quantities up to 5 MLD would necessarily escape detection by the methods employed, since the injection of at least 5 MLD into rabbit fetuses is necessary to provide positive findings.

DISCUSSION

As indicated by the findings in the present study, the exotoxins of *Cl. tetani*, *Cl. oedematiens*, and *C. diphtheriae* do not cross the placental barrier despite the fact that relatively large amounts may be demonstrated in the circulating maternal blood. Some qualification may be made relative to the negative findings inasmuch as there is the remote possibility that transplacental passage could have occurred in such minute amounts as to escape detection. However, observations on fetuses receiving direct inoculation of graded doses of tetanus toxin show that the technics employed can reveal placental transfer of as little as 5 to 10 (mouse) MLD.

These findings cast some doubt on the direct action of bacterial toxin as an influence on the growth and development of the fetus. Quite obviously, it is conceivable that abnormal metabolic by-products of maternal disease may indirectly affect the fetus. Such a possibility is lent credence by the findings of

Ueda who showed that a variety of bacterial toxins, namely streptococcal, staphylococcal, typhoid, and diphtheria, caused contraction of placental blood vessels.[14] These observations led the author to conclude that, as a consequence, disturbances in fetal metabolism might ensue.

SUMMARY

1. The exotoxin of *Cl. tetani* failed to cross the placenta of rabbits, guinea pigs, and rats in detectable amounts.

2. Similar tests for the transplacental passage of *Cl. oedematiens* in rabbits, and *C. diphtheriae* in rabbits and guinea pigs were, likewise, negative.

3. The intracardial, intraperitoneal, and intraplacental administration of dextran and hyaluronidase failed to enhance the passage of tetanus toxin into the fetuses of rabbits.

REFERENCES

1. Klautsch, A.: Die acuten Infectionskrankheiten in ihrer wirkung auf die Schwangerschaft, *München. med. Wchnschr.* **41**: 1049, 1894.
2. Diddle, A. W., and Stephens, R. L.: Typhoid Fever in Pregnancy. Probable Intrauterine Transmission of the Disease, *Am. J. Obst. & Gynec.* **38**: 300, 1939.
3. McCord, J. R.: A Probable Case of Direct Intrauterine Transmission of Tuberculosis From Mother to Baby, *Am. J. Obst. & Gynec.* **19**: 826, 1930.
4. Ballantyne, J. W.: Congenital Measles, With Notes of a Case, *Arch. Pediat.* **10**: 301, 1893.
5. Grindon, J.: An Apparent Case of Varicella in Utero, *J. Cutaneous Diseases* **19**: 237, 1901.
6. Eastman, N.: William's Obstetrics, ed. 10, New York, 1950, Appleton-Century-Crofts, Inc., p. 190.
7. Schmidlechner, C.: Uebergang der Toxine von der Mutter auf die Frucht, *Ztschr. f. Geburtsh u. Gynäk.* **52**: 377, 1904.
8. Mouriquand, G., Leulier, A., and Sedallian, P.: Sur l'arret de l'intoxication diphterique par le placenta, *Compt. rend. Acad. sc.* **190**: 454, 1930.
9. Nattan-Larrier, L., Ramon, G., and Grasset, E.: Recherches sur le passage des toxines et des antitoxines à travers le placenta, *Compt. rend. Soc. biol.* **96**: 241, 1927.
10. Robinson, D., Hardy, P., and Hellman, L.: The Effect of Diphtheria on Pregnancy, With a Report of Five Cases, *Am. J. Obst. & Gynec.* **53**: 1029, 1947.
11. Beck, A. C., and Rosenthal, A. H.: Obstetrical Practice, ed. 6, Baltimore, 1955, The Williams & Wilkins Co., p. 548.
12. Greenhill, J. P.: Obstetrics, ed. 11, Philadelphia, 1955, W. B. Saunders Co., p. 434.
13. Flexner, L. B., and Gellhorn, A.: The Comparative Physiology of Placental Transfer, *Am. J. Obst. & Gynec.* **43**: 965, 1942.
14. Ueda, K.: Bacterial Toxins on Blood Vessels of Human Placenta, *Japanese J. Obst. & Gynec.* **14**: 502, 1931.

FUSIFORMS IN THE INFANT MOUTH

VALERIE HURST, PH.D.

College of Dentistry, University of California Medical Center, San Francisco, Calif.

LITTLE is known of the chronological sequence in which oral microorganisms become established. The aerobic bacteria present during the first week of life have been described by a number of workers, but the occurrence of obligate anaerobes at this age has not been adequately investigated. The oral flora during the later months, after the infant has left the hospital, has seldom been studied. Textbooks state that it undergoes a definite change when the primary teeth begin to erupt.[1, 2] Anaerobic organisms such as fusiforms, spirochetes, and the ill-defined leptothrix group are said to make their first appearance with the development of the gingival crevice. Yet the data contained in the literature are hardly sufficient to justify this statement. The hypothesis seems to have had its origin in the early publication of Lewcowicz[3] who described the oral flora of 2 ill, 1 dead, and 2 well infants and commented that strict anaerobes probably were found only in older mouths because of protected areas and symbiosis. Later Braïlovsky-Lounkevitch[4] deducted that leptothrix, spirochetes, vibrios, etc., appeared with tooth eruption because they were common in the mouths of children aged 1 to 14, but rare in the mouths of newborn infants. Kostečka[5] claimed to have observed anaerobes to appear after tooth eruption and to disappear in the edentulous mouth, but he gave no information on the number of mouths observed, nor his methods. Andre[6] concluded that fusiforms, actinomycetes, and leptothrix appeared with tooth eruption because he cultivated them from 5 of 8 infants aged 7 to 9 months, but not from 26 infants between 8 days and 6 months old. This study, like that of Braïlovsky-Lounkevitch, gave only indirect evidence that the gingival crevice is essential for the growth of anaerobes. It is conceivable that some anaerobes may grow symbiotically with some of the oral aerobes in the absence of the gingival crevice. Pratt[7] reported that oral fusiforms grow in ordinary infusion broth incubated aerobically if other oral bacteria are present simultaneously in the tube. The possible occurrence of fusiforms in the infant mouth prior to dentition was therefore reinvestigated during a recent study on the establishment of the upper respiratory flora.[8]

METHOD

The mouths of 69 babies between 1 day and 8 months old were examined for fusiforms. These babies included newborn infants in a London hospital nursery, foundlings in the Coram Nursery, and babies attending the Child Welfare clinics in London. All were in good health at the time of the examination. Swabs taken from the gingiva, tongue, and buccal mucosa were either

Received for publication June 18, 1956.

(1) rinsed in sterile broth which was inoculated into Robertson's chopped meat medium within a few hours, or (2) inoculated directly into the chopped meat. At intervals between the second to seventh day of incubation, Gram-stained films of the chopped meat medium were examined. When time permitted, subculture of any fusiforms seen in the film was attempted. For this purpose blood agar plates containing either gentian violet 1:20,000, or methyl violet 1:20,000 plus 0.1 per cent cysteine hydrochloride, were incubated anaerobically for 4 to 7 days.

RESULTS

The results obtained are shown in Tables I and II. Fusiforms were cultivated from the mouths of 3 newborn babies aged 5, 6, and 9 days, respectively. From 1 newborn child, they were successfully isolated on an anaerobic plate (Table II). These were Gram-negative rods, 10 μ to 15 μ in length, often with sharply pointed ends, and appeared quite typical. Similar organisms were cultivated from 5 of 16 babies under 2 months old, as well as from nearly all of the babies over 2 months old. In some cases very long Gram-negative filamentous forms were obtained in the chopped meat medium. Subculture of either morphologic type was not always successful (Table II). The colonies varied greatly in appearance, but in general corresponded to those described in the literature as reviewed by Hoffman.[9] They contained Gram-negative cells varying from sharply pointed rods to long slender filaments. All were strict anaerobes, failing to grow on aerobic plates of the same medium.

TABLE I

PRIMARY CULTIVATION OF FUSIFORMS IN CHOPPED MEAT MEDIUM

DENTITION	AGE OF INFANTS	INFANTS		PER CENT POSITIVE
		EXAMINED	POSITIVE	
None	1-10 days	32	3	9
	2 wk.-2 mo.	16	5	31
	2-5 mo.	15	14	94
1 to 3 incisors	6-8 mo.	6	6	100

TABLE II

SUBCULTURE AND ISOLATION OF FUSIFORMS ON ANAEROBIC PLATES

DENTITION	AGE OF INFANTS	CHOPPED MEAT BROTH		PER CENT POSITIVE
		SUBCULTURED	POSITIVE	
None	1-10 days	2	1	58
	2 wk.-2 mo.	5	2	
	2-5 mo.	12	8	
1 to 3 incisors	6-8 mo.	3	3	100

DISCUSSION

During the observations on oral bacteria other than fusiforms it was found that coliforms were common in the infant mouth.[8] Some coliform strains, when grown under semi-anaerobic conditions in liquid media, tended to produce long

filamentous forms. The author now believes that Gram-negative filaments, cultivated with the mixed oral flora in liquid media, should be considered fusiforms only presumptively, unless typical anaerobic colonies can be isolated from these cultures, or coliforms are demonstrated to be absent in aerobic subcultures. Unfortunately, at the time this investigation on fusiforms was being made, the prevalence of coliforms in the mouths of many infants was not realized. Therefore, it is conceivable that some of the filamentous "fusiforms" seen in mixed culture in the chopped meat medium, but not subcultured (Table I), may have been coliforms. That mistaken identity occurred frequently seems improbable. Moreover, there can be no doubt about the colonies isolated anaerobically from 58 per cent of the babies in whom dentition was absent, nor from those with teeth (Table II).

SUMMARY

Gram-negative rods and filaments, believed to be fusiforms, were cultivated almost invariably from the mouths of babies 2 to 5 months old, and sometimes from younger ones, including newborn infants. Typical anaerobic colonies of fusiforms were isolated from the mouths of 58 per cent of 18 babies without dentition, as well as from those with teeth. The gingival crevice is not an essential habitat for the oral fusiforms, as stated in the literature.

The author is indebted to the late Professor Sir Alexander Fleming and Professor Robert Cruickshank for generously extending the laboratory facilities of the Wright-Fleming Institute of Microbiology for the completion of this study, as well as to Professor F. C. Wilkinson for his interest.

REFERENCES

1. Appleton, J. T. L.: Bacterial Infection, With Special Reference to Dental Practice, ed. 4, London, 1950, Henry Kimpton, pp. 409-410.
2. Rosebury, T.: The Bacteriology of Mucous Membranes in Bacterial and Mycotic Infections of Man, ed. 2, ed. Dubos, R., Philadelphia, 1952, J. B. Lippincott Co., pp. 694-695.
3. Lewcowicz, X.: Recherches sur la flore microbienne de la bouche des nourissons, *Arch. Méd. exp.* **13**: 663, 1901.
4. Braïlovsky-Lounkevitch, Z. A.: Contribution a l'etude de la flore microbienne habituelle de la bouche normale, *Ann. Inst. Pasteur* **29**: 379, 1915.
5. Kostečka, F.: Relation of the Teeth to the Normal Development of Microbial Flora in the Oral Cavity, *D. Cosmos* **66**: 927, 1924.
6. Andre, H.: Beitrage zur anaeroben Mundflora, *Dtsch. zahnärtztl. Wschr.* **19**: 433, 1934.
7. Pratt, J. S.: On the Biology of *B. fusiformis*, *J. Infect. Dis.* **41**: 461, 1927.
8. Hurst, V.: Studies on the Bacterial Flora of the Infant Upper Respiratory Tract, Ph.D. Thesis, University of London, 1954.
9. Hoffman, F.: Bacteriology of the Fusobacteria: A Review, *Oral Surg, Oral Med., & Oral Path.* **5**: 1077, 1952.

THE EXTRA-OSSEOUS INNERVATION OF THE GINGIVAE

DIPYA MONGKOLLUGSANA, M.S., D.M.D.,* AND LINDEN F. EDWARDS, PH.D.
Department of Anatomy, The Ohio State University, Columbus, Ohio

ALTHOUGH the literature, bearing upon the innervation of a tooth and its periodontium, as demonstrated microscopically, is rather voluminous, information concerning the total number of nerves that are involved in innervating all of the mandibular and maxillary teeth and their supporting structures, especially the gingivae, is scant.

Several investigators, notably Stewart and his co-workers,[18, 39-41] have demonstrated by means of histologic preparations that the supporting structures of a tooth are innervated by intra-osseous and extra-osseous nerves. The term intra-osseous refers to the alveolar or dental nerves which course within the mandible and maxillae. According to these investigators, fibers of these nerves supply not only the dental pulp but also the periodontal membrane, the surrounding alveolar process, and the gingiva. By the term extra-osseous nerves is meant those nerves which are situated external to the mandible and maxillae on their buccolabial and palatolingual aspects and from which, according to these investigators, fibers can be traced microscopically to the gingiva, alveolar process, and periodontal membrane. Thus, according to these authors, and, as shown in an illustration in one of their papers,[18] the gingiva adjacent to any tooth is innervated by nerve fibers from 3 different sources, namely, from the intra-osseous or alveolar nerve, from an extra-osseous nerve situated on the buccolabial aspect of the jaw, and from an extra-osseous nerve located on the palatal or lingual aspect. Inasmuch as these authors were concerned primarily with the distribution of the nerve fibers from these 3 sources as observed microscopically, their studies did not include an identification of the extra-osseous nerves involved.

The objective of the present paper is to report the results of a study of the extra-osseous nerves which are involved in innervating the gingivae as determined by careful, tedious, gross dissection.

REVIEW OF LITERATURE

Prior to the inauguration of this study, 10 standard textbooks of human anatomy and 5 texts of local anesthesia were examined with the view to ascertaining what information they provide concerning the nerve supply of the gingivae. The most recent editions of 3 standard American texts of human

This paper is based on a thesis submitted in partial fulfillment of the requirements of the Master of Science degree, Graduate School, The Ohio State University, by the senior author.

Received for publication June 6, 1956.

*Fulbright Fellow: Bangkok, Thailand. Present address: Bangkok, Thailand.

anatomy[12, 13, 20] and of 2 standard British texts[8, 14] were selected for the survey, including older editions, 2 of which are domestic[11, 27] and 1 foreign.[29] Also examined were 2 recent editions of texts devoted exclusively to the anatomy of the head and neck.[32, 35] The 5 textbooks of local anesthesia were selected at random.[9, 19, 21, 36, 42]

The results of the textbook survey are tabulated in Tables I and II. Although unanimity of opinion concerning the total number of nerves involved is lacking, it can be seen from the tables that 10 nerves are claimed by 1 or more of the texts to innervate the gingivae. Since 10 standard textbooks of human anatomy were surveyed, the frequency or percentage of agreement of these texts concerning any 1 nerve can readily be calculated and is indicated in the right-hand column of Table I, the range varying from 20 to 100 per cent. The total per cent of the 10 nerves involved according to each text is indicated in the bottom line of the table, the range varying from 30 to 80 per cent. The percentage of frequencies of agreement in the textbooks of local anesthesia concerning these 10 nerves is shown in Table II.

It is quite evident, therefore, from this survey of authoritative textbooks of human anatomy and of local anesthesia, that none of them contain complete information concerning the innervation of the gingivae.

A review of anatomic and dental journals published in the English language likewise fails to reveal any reports of a comprehensive coverage of the subject. Several papers, which deal with failures in local anesthesia, not only offer suggestions as to methods of obtaining successful results but also assume the presence of aberrant collateral innervation of the teeth and their supporting structures. Thus, it has been suggested[10, 15, 30] that pain fibers to these structures may accompany sympathetic fibers in the periarterial plexus on the alveolar arteries as well as on other arteries supplying the jaws, a suggestion incidentally that merits investigation. Several authors postulate, without furnishing verifiable evidence, that the nasopalatine nerves as they descend through the incisive canals supply twigs to the pulps of the maxillary first incisors (Cook,[6] Hofer, as quoted by Sicher,[34] Phillips,[25] and Phillips and Maxmen,[26] a postulation that has been challenged (Olsen, Teuscher, and Vehe,[24] and Sicher[34]).

A much debated question is whether or not there is an overlap of nerve fibers of the 2 inferior alveolar and lingual nerves across the symphysis menti, some authors contending such an overlap exists,[37, 41] while others (Oliver,[23] Mileff, as quoted by Stewart[39]) denied such an overlap. Some authors describe the occasional occurrence of a retromolar branch of the inferior alveolar nerve which supplies the mucosa of the retromolar triangle of the mandible and which may enter a foramen in that area to furnish fibers to the lower molar teeth (Dieck and Fugita, as quoted by Batson,[3] and Angelman[2]). Novitsky[22] and Schumacher (quoted by Sicher[34]) claimed they found twigs from the mylohyoid nerve entering a foramen on the lingual surface of the mandible below the genial tubercles and Angelman[2] mentioned that twigs from the lingual nerve occasionally enter nutrient canals in the same area.

TABLE I

INNERVATION OF GINGIVAE ACCORDING TO TEN HUMAN ANATOMY TEXTBOOKS

	CUNNINGHAM (1951)	GERRISH (1899)	GRANT (1952)	GRAY (AM. '54)	GRAY (ENG. '46)	MORRIS (1953)	PIERSOL (1930)	QUAIN (1909)	SHAPIRO (1954)	SICHER (1952)	PER CENT
Infraorbital			x						x		20
Ant. Sup. Alv.						x		x	x	x	40
Mid. Sup. Alv.						x		x	x	x	40
Post. Sup. Alv.	x	x	x	x	x	x	x	x	x	x	100
Nasopal.			x					x			20
Great. pal.		x	x	x	x	x	x	x			70
Mental			x							x	20
Inf. Alv.		x	x		x	x	x	x	x	x	80
Long Buccal	x		x	x	x				x	x	60
Lingual	x	x	x	x	x		x	x	x	x	90
Per cent	30	40	80	40	50	50	40	70	70	70	

TABLE II

INNERVATION OF GINGIVAE ACCORDING TO 5 TEXTBOOKS ON BLOCK ANESTHESIA

	FISCHER AND GROSSMAN	MEAD	NEVIN AND PUTERBAUGH	SMITH	THOMA	PER CENT
Infraorbital					x	20
Ant. Sup. Alv.	x		x	x		60
Mid. Sup. Alv.	x		x	x		60
Post. Sup. Alv.	x	x	x	x	x	100
Nasopal.	x		x			40
Great. pal.	x		x	x		60
Mental	x		x	x	x	80
Inf. Alv.	x			x	x	60
Long Buccal	x	x		x	x	80
Lingual		x	x	x	x	80
Per cent	80	30	70	80	60	

One of the most unusual claims, that has been made, is that occasionally a twig of the nervous cutaneous coli, which is a branch of the cervical plexus, enters the mandible through a tiny foramen situated near the inferior border on its lingual aspect between the premolar teeth.[1, 2, 25, 38] Several authors[3, 34] questioned this claim. Sicher[34] stated flatly that "such a nerve does not exist, as careful observations on 50 cadavers have proved," and he contended that this erroneous claim is based merely on the presence of the tiny foramen on the mandible. Shiller and Wiswell[33] investigated the frequency of occurrence of this foramen on 126 mandibles and found it on 63.5 per cent of them. These authors hazard the opinion that it presents the possibility of collateral innervation.

It is of interest to note that, according to Stewart and Lewinsky,[40] "the actual source of the nerves of the gum is still in doubt," and, as recent as 1950, Sicher[34] claimed, "there is still no certainty as to all aspects of the nerve supply of the upper jaw . . . also in the nerve supply to the mandible several problems await solution."

OBSERVATIONS

Inasmuch as the objective of this study was to determine by gross dissection the extra-osseous nerve supply of the gingivae, no effort was made to verify the observations of other investigators relative to the distribution of fibers of the intra-osseous nerves of the mandible and maxilla to the gingivae. Moreover, no attempts were made to trace fibers of the extra-osseous nerves to their terminal endings in the gingivae, since this procedure, involving microtechnic, was beyond the scope of this study; it was felt that it could safely be assumed that at least some of the fibers within twigs traceable grossly to the gingivae terminate in the latter without any necessity of demonstrating their terminations microscopically, especially in view of the findings of other investigators.

Taking our cue from the list of extra-osseous nerves compiled in Tables I and II, the jaws of several cadavers were carefully dissected with the view to determining whether any twigs of these nerves could be traced to the gingivae of the mandible and maxilla, meanwhile observing closely whether or not

twigs from any other source could be detected entering the gingivae. Difficulties were frequently encountered in attempting to trace filaments from certain of these nerves to the gingiva, especially the palatal, due to the abundance and toughness of the connective-tissue fibers of the mucoperiosteum, in which the nerve filaments are embedded. Also the slenderness of nerve filaments often hampered their successful dissection and preservation. However, a sufficient number of them, from each nerve involved, was eventually isolated and preserved in order to enable us to obtain a permanent photographic record of their distribution.

The posterior superior alveolar nerve arises from the maxillary nerve within the pterygopalatine fossa and descends upon the infratemporal surface of the maxilla where it gives off several branches. Some of these enter the alveolar canals on this surface of the maxilla to help supply the mucous membrane of the maxillary antrum and to innervate the molar teeth (with the exception of the mesiobuccal root of the first molar) and their supporting structures. Other branches (Fig. 1, *A*) can readily be traced downward to the buccal gingiva at the level of the molar and premolar teeth.

The infraorbital nerve is that portion of the maxillary nerve which traverses the infraorbital canal and emerges onto the face by passing through the infraorbital foramen. It divides into 3 terminal branches, palpebral to the lower eyelid, nasal to the side of the nose, and labial to the skin and mucous membrane of the upper lip and to the labial gingiva of the incisor and canine teeth (Fig. 1, *A*).

The nasopalatine nerve takes its origin from the sphenopalatine (Meckel's) ganglion which is suspended from the maxillary nerve within the pterygopalatine fossa. It enters the nasal fossa through the sphenopalatine foramen and descends upon the nasal septum to the incisive canal which opens onto the hard palate as the incisive foramen. The nerve emerges through the median subdivision (foramen of Scarpa) of the incisive foramen onto the hard palate. With difficulty, small filaments can be traced into the palatal gingiva of the incisor and canine teeth (Fig. 1, *B*).

The greater (anterior) palatine nerve likewise takes origin from the sphenopalatine ganglion. It descends through the pterygopalatine canal which opens onto the hard palate as the greater palatine foramen. Emerging from this foramen, it passes anteriorly within a groove in the hard palate and as it does so it gives off several small filaments, some of which pass medialward to the mucous membrane of the hard palate and others extend lateralward to the palatal gingiva of the molar and premolar teeth (Fig. 1, *B*).

The long buccal or buccinator nerve is the terminal branch of the anterior division of the mandibular nerve. It arises from the latter nerve within the infratemporal fossa deep to the external pterygoid muscle, between the 2 heads of which it emerges to enter the pterygomandibular space. It passes antero-inferiorly on the superficial surface of the internal pterygoid muscle, penetrates the temporal tendon, and enters the cheek at about the level of the

occlusal plane of the lower molars. It breaks up into several branches most of which are distributed to the skin and mucous membrane of the cheek. Just as the main trunk enters the cheek it gives off a branch which follows the buccal shelf of the mandible and gives off several twigs to the buccal gingiva of the molars and sometimes as far anteriorly as the first premolar (Fig. 1, *C*).

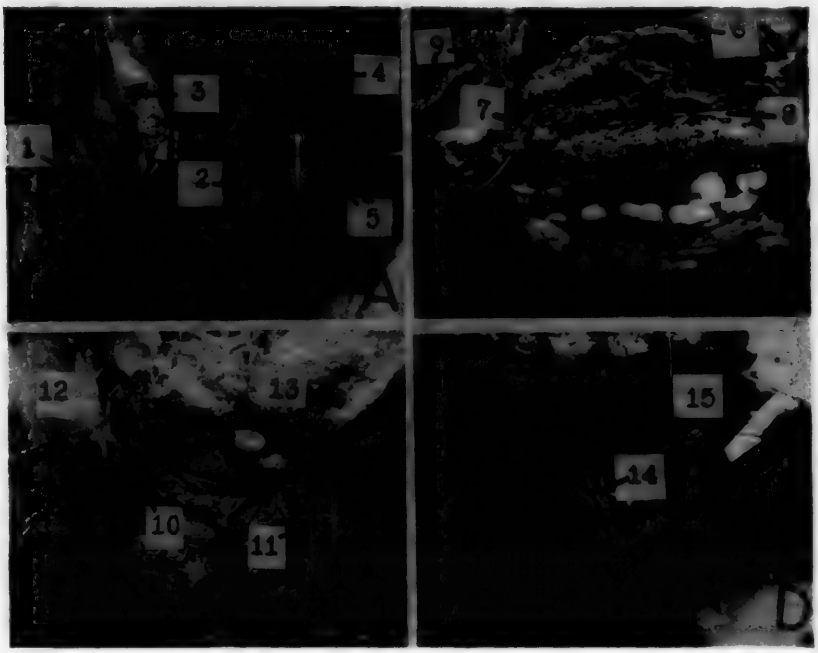


Fig. 1.—Dissections of the extra-osseous nerves supplying the maxillary and mandibular gingivae.

A, Anterolateral aspect of the maxilla, showing the distribution of the posterior superior alveolar (*1*) and infraorbital (*2*) nerves to the buccolabial gingiva. *3*, zygomatic process. *4*, nasal bone. *5*, upper lip everted.

B, Midsagittal section of the palate (*6*) showing the distribution of the nasopalatine (*7*) and greater (anterior) palatine (*8*) nerves to the palatal gingiva. *9*, upper lip.

C, Lateral aspect of the mandible showing the distribution of the long buccal (*10*) and mental (*11*) nerves to the buccolabial gingiva. *12*, ramus of mandible. *13*, cheek everted.

D, Lingual aspect of the body of the mandible showing twigs from the lingual nerve (*14*) to the lingual gingiva (*15*).

The mental nerve is a branch of the inferior alveolar nerve, from which it arises within the mandibular canal, and emerges through the mental foramen. It very soon divides into many branches, most of which are distributed to the skin and mucous membrane of the lower lip and to the skin over the chin. However, with careful dissection several twigs can be traced into the labial gingiva of the first and second incisor and canine teeth and oftentimes of the first and second premolars (Fig. 1, *C*).

The lingual nerve is one of the branches of the posterior division of the mandibular nerve, taking its origin from the latter deep to the external pterygoid muscle, at the lower border of which it enters the pterygomandibular

space anterior to the inferior alveolar nerve. It passes antero-inferiorly on the lateral surface of the internal pterygoid muscle and enters the sublingual space just lingual to the roots of the third molar tooth where it lies immediately deep to the mucous membrane of the floor of the mouth (alveololingual sulcus). It passes anteriorly along the side of the tongue to the tip of the latter and as it does so it gives off filaments to the mucous membrane on the anterior two thirds of the tongue, a sublingual branch to the sublingual gland, and 2 or more branches to the submaxillary ganglion. Exercising care, one can demonstrate twigs which pass laterally to enter the lingual gingiva (Fig. 1, *D*) as far anteriorly as the symphysis menti. One of these twigs, or a twig from the sublingual branch, is often seen to enter the foramen just superior to the genial tubercles or mental spines in company with a branch of the sublingual artery and corresponding vein. The twigs from the lingual nerve to the gingiva are invariably destroyed when the sublingual space is dissected by an extra-oral approach from below and are best observed, therefore, when this space is dissected intra-orally, especially if the mucous membrane of the floor of the mouth is carefully removed from above.

In spite of diligent search for twigs to the gingivae from any other possible extra-osseous nerves, none were detected. Although the mylohyoid nerve traverses the submaxillary or digastric triangle on the superficial surface of the mylohyoid muscle it was seen occasionally to give off twigs which penetrate the latter muscle and appear on its deep surface. However, none of these twigs were observed to enter the lingual gingiva or the foramen superior to the mental spines. According to Gray's Anatomy[13] (p. 999), the mylohyoid nerve may communicate with the lingual nerve. No evidence of such communication was found in this study. However, such a communication or the presence of twigs of the mylohyoid nerve to the mandibular gingiva should not be too surprising, inasmuch as the mylohyoid nerve is a branch of the inferior alveolar nerve and, therefore, might conceivably contain pain fibers of the latter nerve which are rerouted via the mylohyoid nerve to the supporting structures of the mandibular teeth.

CONCLUSIONS

The conclusions arrived at from this study concerning the extra-osseous nerve supply of the gingivae are as follows:

1. The palatal gingiva of the maxillary incisors and canines is supplied by the nasopalatine nerve.
2. The palatal gingiva of the maxillary premolars and molars is supplied by the greater (anterior) palatine nerve.
3. The labial gingiva of the maxillary incisors and canines is supplied by the labial branch of the infraorbital nerve.
4. The buccal gingiva of the maxillary premolars and molars is supplied by the posterior superior alveolar nerve.
5. The lingual gingiva of all the mandibular teeth is supplied by the lingual nerve.

6. The labial gingiva of the mandibular incisors and canines, including in some instances the first and even the second premolar, is supplied by the mental nerve.

7. The buccal gingiva of the mandibular molars and sometimes the second and even the first premolar is supplied by the long buccal nerve.

Evidence is furnished by the illustrations in Fig. 1.

SUMMARY

Inasmuch as there is lack of agreement in standard textbooks of human anatomy as to which nerves innervate the supporting structures of teeth, especially the gingivae, the present study was undertaken to determine by means of gross dissection the extra-osseous nerve supply of the maxillary and mandibular gingivae.

By extra-osseous nerves is meant those nerves situated on the buccolabial and palatolingual aspects of the maxilla and mandible as opposed to the alveolar (dental) nerves which are (intra-osseous) within the bodies of these bones.

The nerves involved were found to be the nasopalatine and greater (anterior) palatine to the palatal gingiva of the maxillary teeth, the infraorbital to the labial gingiva of the maxillary incisors and canines, the posterior superior alveolar (dental) to the buccal gingiva of the maxillary molars and premolars, the lingual to the lingual gingiva of the mandibular teeth, the mental to the labial gingiva of the mandibular incisors, and canine and the long buccal to the gingiva of the mandibular molars and premolars.

REFERENCES

1. Adams, F. R.: Control of Pain in Operative Dentistry, *Bul. D. Soc. N. Y.* **5**: 7, 1938.
2. Angelman, J.: Failures in Local Anesthesia, *Brit. D. J.* **80**: 220-223, 1946.
3. Batson, O. V.: The Gross Anatomy of the Innervation of the Teeth, Jaws, Face and Related Parts, in *Appleton's* Control of Pain in Dental Practice, Chapt. I, Philadelphia, 1940, J. B. Lippincott Co.
4. Bradlaw, R.: The Innervation of the Teeth, *Proc. Roy. Soc. Med.* **29**: 507-518, 1936.
5. *Idem:* Histology and Histopathology of Dental Innervation, *ibid.* **32**: 1042-1053, 1939.
6. Cook, W. A.: The Nerve Supply to the Maxillary Incisors, *Modern Dent.* **17**: 9-18, 1950.
7. *Idem:* The Anterior Superior Alveolar Nerve and Its Control With Local Anesthetics, *D. Items Interest* **72**: 1021-1028, 1950.
8. Cunningham, D. J.: Text-book of Anatomy, ed. 9, edited by J. C. Brash, London, 1951, Oxford University Press.
9. Fischer, G.: Local Anesthesia in Dentistry, Philadelphia, 1933, Lea & Febiger.
10. Frazier, C. H.: Pain Phenomena of the Face, Their Origin and Treatment, With Special Reference to Trigeminal Neuralgia, *Am. J. M. Sc.* **169**: 469-476, 1925.
11. Gerrish, F. H.: Text-book of Anatomy, Philadelphia, 1899, Lea Bros. & Co.
12. Grant, J. C. B.: Method of Anatomy, ed. 5, Baltimore, 1952, Williams and Wilkins Co.
13. Gray, H.: Anatomy of the Human Body, ed. 26, edited by C. M. Goss, Philadelphia, 1954, Lea & Febiger.
14. *Idem:* Gray's Anatomy, Descriptive and Applied, edited by T. B. Johnson and J. Whillis, London, 1938, Longmans, Green & Co.
15. Helson, H., and Frazier, C. H.: The Part Played by the Sympathetic System as an Afferent Mechanism in the Region of the Trigeminus, *Brain* (Part I) **55**: 114-121, 1932.
17. Jones, F. W.: The Anterior Superior Alveolar Nerve and Vessels, *J. Anat.* **73**: 583-591, 1939.
18. Lewinsky, W., and Stewart, D.: An Account of Our Present Knowledge of the Innervation of the Teeth and Their Related Tissues, *Brit. D. J.* **65**: 687-700, 1938.
19. Mead, S. V.: Anesthesia in Dental Surgery, St. Louis, 1951, C. V. Mosby Company.

20. Morris' Human Anatomy, ed. 11, edited by J. P. Schaeffer, New York, 1953, The Blakiston Co.
21. Nevin, M., and Puterbaugh, P. G.: Conduction, Infiltration and General Anesthesia in Dentistry, Rev. ed. 5, Brooklyn, 1948, Dental Items of Interest Publishing Co.
22. Novitsky, J.: Sensory Nerves and Anesthesia of the Teeth and Jaws, *Modern Dent.* **5**: 5-10, 1938.
23. Oliver, E.: The Inferior Dental Canal and Its Nerve in the Adult, *Brit. D. J.* **49**: 356-358, 1927.
24. Olsen, N. H., Teuscher, G. W., and Vehe, K. L.: A Study of the Nerve Supply to the Upper Anterior Teeth, *J. D. Res.* **34**: 413-420, 1955.
25. Phillips, W. H.: Anatomic Considerations in Local Anesthesia, *J. Oral Surg.* **1**: 112-121, 1943.
26. Phillips, W. H., and Maxmen, H. A.: The Nasopalatine Block Injection as an Aid in Operative Procedures for Maxillary Incisors, *Am. J. Orthodont. & Oral Surg.* **27**: 426-434, 1941.
27. Piersol, G. A.: Human Anatomy, ed. 9, Philadelphia, 1930, J. B. Lippincott Co.
28. Prothero, J. H.: Innervation of the Oral Cavity and Contiguous Parts, *J. A. D. A.* **10**: 1111-1113, 1923.
29. Quain's Elements of Anatomy, ed. 11, edited by Shafer et al., Vol. III, London, 1909, Longmans, Green & Co.
30. Seldin, H. M.: Painless Dentistry: Fact or Fable, *D. Cosmos* **68**: 793-795, 1926.
31. *Idem:* Practical Anesthesia for Dental and Oral Surgery, ed. 3, Philadelphia, 1947, Lea & Febiger.
32. Shapiro, H. H.: Maxillofacial Anatomy, Philadelphia, 1954, J. B. Lippincott Co.
33. Shiller, W. R., and Wiswell, O. B.: Lingual Foramina of the Mandible, *Anat. Rec.* **119**: 387-390, 1954.
34. Sicher, H.: Aspects in the Applied Anatomy of Local Anesthesia, *Internat. D. J.* **1**: 70-82, 1950-51.
35. *Idem:* Oral Anatomy, ed. 2, St. Louis, 1952, C. V. Mosby Company.
36. Smith, A. E.: Block Anesthesia and Allied Subjects, St. Louis, 1921, C. V. Mosby Company.
37. Starkie, C., and Stewart, D.: The Intramandibular Course of the Inferior Dental Nerve, *J. Anat.* **65**: 319-323, 1931.
38. Steinfelder, L. J.: Problems in Local Anesthesia, *D. Outlook* **23**: 169-172, 1936.
39. Stewart, D.: The Innervation of the Dental Tissues and Its Importance in Regional Anesthesia, *Brit. D. J.* **53**: 277-284, 1932.
40. Stewart, D., and Lewinsky, W.: Comparative Study of Innervation of the Gum, *Proc. Roy. Soc. Med.*, London **32**: 1054-1062, 1939.
41. Stewart, D., and Wilson, S. L.: Regional Anesthesia and Innervation of the Teeth, *Lancet* **215**: 809-811, 1928.
42. Thoma, K. H.: Oral Anesthesia, Boston, 1914, Ritter and Flebbe.
43. Van der Sprenkel, H. B.: Microscopical Investigation of the Innervation of the Tooth and Its Surroundings, *J. Anat.* **70**: 233-241, 1936.

THE RELATION OF SALIVARY SODIUM, POTASSIUM, SOLIDS AND ASH CONCENTRATION TO DENTAL CARIES EXPERIENCE IN CHILDREN, 5 TO 6 AND 12 TO 14 YEARS OF AGE

I. ZIPKIN, F. A. BULLOCK,* AND N. MANTEL**

National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

NUMEROUS attempts have been made to relate the chemical composition of whole saliva to dental caries experience. Many of these studies, reviewed by Karshan[1] and Dewar,[2] as well as by others,[3-12] have failed to reveal any unequivocal correlation between the cations, calcium[3-9] and ammonium,[10-12] and dental caries. The concentration of magnesium was found to be higher in caries-resistant than in caries-active individuals.[9] On the basis of previous limited observations, the salivary sodium concentration was unrelated to caries experience.[13, 14] The potassium concentration of saliva of caries-immune individuals was reported higher,[13] lower,[14] or the same[15] as that of caries-active subjects.

The development of a simple flame photometer by Barnes, Richardson, Berry, and Hood,[16] for the determination of sodium and potassium, furnished considerable impetus for the investigation of the salivary content of these ions under a variety of systemic conditions.[17-23]

The purpose of the present investigation was to re-evaluate the relation between the salivary sodium-potassium ratio and dental caries experience in a large number of cases.

METHODS

Using a mouth mirror and explorer, the number of def and DMF teeth was determined on 238 children aged 5 to 6 years and on 272 children aged 12 to 14 years residing in Grand Rapids, Mich.† Samples of whole, mixed, paraffin-stimulated saliva collected between 10 and 11 A.M. or 2 and 3 P.M. were analyzed for sodium and potassium using the Process and Instruments internal standard flame photometer.[24]

Five milliliters of saliva were transferred into a platinum crucible, dried to constant weight at 105° C. for 2 hours, placed in a cold furnace, and ashed at 550° C. for 1 hour. Five milliliters of 1 N HCl were added, the sample placed on a steam bath for 30 minutes, filtered through Whatman No. 1 filter paper, washed with hot deionized water,‡ allowed to cool, and made up to 50.0 ml.

Received for publication Sept. 10, 1956.

*Formerly with the National Institute of Dental Research, now with the National Heart Institute.

**National Cancer Institute.

†Taken from examinations made by NIDR staff in Grand Rapids, Mich., in 1950.

‡Deionized water was used throughout.

Twenty milliliters were placed in a 100-ml. volumetric flask, 3.0 ml. of 1 N LiCl added as internal standard, and the samples diluted to volume. Thus, the samples introduced into the flame photometer represented a 50-fold dilution of the original saliva.

Standard curves were prepared prior to analysis of each group of 20 samples, although repeated determinations of both ashed and unashed standards showed excellent agreement. A 25 ppm sodium or potassium solution containing 3.0 meq. per 100 ml. of LiCl as internal standard was set at a helipot reading of 1,000. Several dilutions of these stock standards gave the straight line functions

$$X = \frac{Y\text{-}100}{36.0} \text{ for sodium; and } X = \frac{Y\text{-}130}{34.8} \text{ for potassium;}$$

where X = concentration of sodium or potassium in ppm, and Y = helipot reading up to a maximum of 1,000.

The interference of calcium, magnesium, phosphate, and sulfate added singly to standard solutions containing 15 ppm Na or K and 3.0 meq. of LiCl per 100 ml. was determined. Sodium and potassium were furnished as the chloride, calcium and magnesium as the carbonate dissolved in 1 N HCl, and phosphorus and sulfur as phosphoric and sulfuric acids, respectively. The analytical grade reagents were NaCl, KCl, $CaCO_3$, and $MgCO_3$; phosphorus was determined colorimetrically and titrimetrically, and sulfur, titrimetrically. The "apparent" concentration of sodium or potassium was determined from a standard curve with 25 ppm Na or K set at a helipot reading of 1,000. Thus, as shown in Table I, the level of interference of various ions was reduced below their maximum concentration reported in the literature[9, 25-28] in the 1:50 dilution of saliva used for analysis.

TABLE I
INTERFERENCE OF VARIOUS IONS ON THE DETERMINATION OF SODIUM AND POTASSIUM

INTERFERING ION	MAXIMUM CONCENTRATION OF ION SHOWING NO INTERFERENCE		MAXIMUM CONCENTRATION OF ION IN 1:50 DILUTION OF SALIVA (LITERATURE) (PPM)
	WITH 15 PPM Na (PPM)	WITH 15 PPM K (PPM)	
K^+	100	—	32
Na^+	—	100	30
Ca^{++}	10	10	0.2
Mg^{++}	10	500	0.04
$PO_4 \equiv$	5	5,000	0.3
$SO_4 =$	10	1,000	0.6

A composite solution containing 15 ppm Na, 15 ppm K, 2 ppm Ca, 1 ppm Mg, 5 ppm P, and 5 ppm S gave the same flame emission density as solutions containing 15 ppm Na and a 15 ppm K, respectively. Hence the simultaneous presence of these elements did not interfere in the determination of sodium and potassium.

An artificial saliva was prepared which simulated whole human saliva as to physical appearance and chemical composition, except that it was essentially free of sodium and potassium (0.6 ppm Na and 0.1 ppm K). The initial pH of 2.0 was adjusted to 7.1 with NH_4OH and CH_3COOH, and the final volume was 500 ml. This artificial saliva was prepared as follows:*

Compound	*Amount*	*Final Concentration (Mg. %)*	*Reference*
Ca as $CaCO_3$ in 1 N HCl	40.0 ml. of 1,000 ppm Ca	8.0	25, 26, 27
Mg as $MgCO_3$ in 1 N NCl	5.0 ml. of 1,000 ppm Mg	1.0	9, 25, 27
P as H_3PO_4	37.5 ml. of 2,000 ppm P	15.0	9, 25, 26, 27
S as H_2SO_4	15.0 ml. of 10,000 ppm S	30.0	27
Protein as gastric mucin	2.0 Gm.	400	26
Urea	75.0 mg.	15	26
Cholesterol	50.0 mg.	10	26
Uric acid	5.0 mg.	1	26

When 4, 8, 12, 16, and 20 ppm sodium and potassium were added to the artificial saliva and carried through the analytical procedure, the recovery was 99 to 104 per cent in each instance.

The flame photometer, therefore, was used for the determination of sodium and potassium in saliva samples of 510 children for whom clinical caries data were available. The caries scores, the sodium and potassium concentration, the sodium-potassium ratio, and the per cent solids and ash are shown in Table II. Least squares multiple regression equations were fitted relating def or DMF to the Na, K, log Na/K, per cent solids and ash. These were fitted with equal regression coefficients, but with differing intercepts for each sex and age category. For def, the age categories were 5 or 6 years, and for the DMF, the categories were 12, 13, or 14 years. For each age, the children were further divided by sex.

TABLE II
THE def AND DMF TEETH IN 5- TO 6- AND 12- TO 14-YEAR-OLD CHILDREN, RESPECTIVELY, AND THE SALIVARY Na, K, Na/K, % SOLIDS AND ASH

AGE (YEARS)	def TEETH	DMF TEETH	Na (MEQ./L.)	K (MEQ./L.)	AVERAGE Na AVERAGE K	SOLIDS (%)	ASH (%)
			Average ± 1 standard error				
5 and 6							
Boys (132)	4.14 ± 0.36	—	19.0 ± 0.8	19.9 ± 0.2	0.95 ± 0.05	0.550 ± 0.008	0.281 ± 0.005
Girls (106)	4.58 ± 0.41	—	17.1 ± 0.8	19.6 ± 0.3	0.87 ± 0.05	0.539 ± 0.009	0.267 ± 0.005
Difference	−0.44 ± 0.54	—	+1.9 ± 1.2	+0.3 ± 0.4	+0.08 ± 0.07	+0.011 ± 0.012	+0.014 ± 0.007
12, 13, 14							
Boys (188)	—	7.21 ± 0.31	23.8 ± 0.8	19.2 ± 0.2	1.24 ± 0.05	0.597 ± 0.007	0.291 ± 0.004
Girls (84)	—	7.36 ± 0.47	19.0 ± 0.7	19.2 ± 0.3	0.99 ± 0.04	0.567 ± 0.009	0.264 ± 0.004
Difference	—	−0.15 ± 0.56	+4.8 ± 1.1	0.0 ± 0.3	+0.25 ± 0.07	+0.030 ± 0.012	+0.027 ± 0.006
			Standard deviations				
5 and 6							
Boys	4.19	—	9.6	2.9	—	0.086	0.055
Girls	4.17	—	8.2	2.9	—	0.091	0.054
12, 13, 14							
Boys	—	4.19	11.2	2.8	—	0.095	0.059
Girls	—	4.31	6.8	2.3	—	0.087	0.037

*Muhler and Swenson[28] had previously reported the preparation of a synthetic saliva which, however, differed substantially in some components from values reported in the literature.[9, 25-27]

The analysis of variance for the multiple regression is shown in Table III.

TABLE III
ANALYSIS OF VARIANCE FOR THE MULTIPLE REGRESSION

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	P
		5 and 6 year olds, def			
Multiple regression	166.81	5	33.36	1.92	Approx. 0.1
Remainder within classes	3934.29	229	17.18		
Total, within classes	4101.10	234			
$R^2 = \frac{166.81}{4101.10} = 0.0407$,		$R = 0.202$			
		12 to 14 year olds, DMF			
Multiple regression	109.87	5	21.97	1.24	Approx. 0.3
Remainder within classes	4630.58	261	17.74		
Total, within classes	4740.45	266			
$R^2 = \frac{109.87}{4740.45} = 0.0231$,		$R = 0.152$			

RESULTS

As shown in Table II, significant sex differences were observed in some of the salivary constituents of the 12- to 14-year-old group. Boys were significantly higher than girls in this age group in their average concentration of sodium ($p < 0.01$), solids ($p < 0.05$) and ash ($p < 0.01$). There was no significant difference in the average potassium concentration, and the significantly higher sodium-potassium ratio ($p < 0.01$) for boys reflects the difference noted in the sodium concentration. The sex differences in the 5- to 6-year-old children were similar in direction to the significant differences found among the older children. None, however, was significant except for a higher concentration of ash in the 5- to 6-year-old boys ($p < 0.05$).

From the data of Table II, it was also possible to compare the sodium, potassium, Na/K ratio, solids and ash between the 2 age groups by sex. Thus, the sodium concentration, the Na/K ratio, and the per cent solids of the 12- to 14-year-old boys were significantly higher than those of the 5- to 6-year-old boys ($p < 0.01$), whereas the potassium concentration was lower ($p < 0.02$). The age changes for girls were in the same direction as the significant age changes for boys. No significant changes were observed in the 2 age groups for the girls, except, however, for an increase in the per cent solids in the 12- to 14-year-old girls ($p < 0.05$).

As shown in Table III, the multiple regression relating the def or DMF to the Na, K, log Na/K, per cent solids and ash was not significant for either age group.

For the def data in the 5 and 6 year olds, the multiple regression accounted for only 4.1 per cent of the total variation within the sex and age categories and for the DMF data in the 12 to 14 year olds, only 2.3 per cent. While some of the individual multiple regression coefficients were significant or suggestive, they clearly indicate that the various salivary constituents play no important role in accounting for the variation in the def or DMF.

DISCUSSION

It will be noted that the variation in the sodium concentration of saliva was 3 to 4 times that of potassium as shown by the respective standard deviations in Table II. The concentration of sodium varied from 2.2 to 68.0 meq./L. and that of potassium from 12.0 to 27.7 meq./L. These values are narrower in range than those reported for sodium of 2.0 to 74.4 meq./L. and potassium of 11.3 to 40.4 meq./L. in whole, paraffin-stimulated saliva.[17, 19, 20, 30-34]

The effect of age on the content of these ions in whole paraffin-stimulated saliva appears to be limited to the report of Niedermeier[35] who stated that the sodium concentration decreased with age, whereas the potassium concentration remained unchanged. Data on "resting" saliva[35-38] in these regards are conflicting. Niedermeier,[35] Becks,[36] and Grad[37] reported an increase in both sodium and potassium in individuals 55 (mean), 8 to 71 and 5 to 86 years of age, respectively. Hungerland, Qwenzlein, and Weber,[38] on the other hand, reported a decrease in these constituents in individuals up to 25 years of age. No previous data appear to be available on the effect of sex on the sodium and potassium content of stimulated saliva. In the present study, males, 12 to 14 years of age, had a higher sodium ($p < 0.01$) and a lower potassium concentration ($p < 0.02$) than males 5 to 6 years of age. No differences were observed in the females. Grad[37] reported that the sodium concentration of unstimulated mixed saliva "tended to be higher" in males than in females, while the potassium concentration was the same. Similar results were found in the present studies for paraffin-stimulated salivas of individuals 12 to 14 years of age.

The concentration of sodium in stimulated whole saliva is reported to be directly proportional to the rate of secretion,[31, 39] whereas the concentration of potassium shows a slight, but statistically insignificant, decrease with an increased rate of flow.[31, 39] Pawan[23] has also shown that the sodium-potassium ratio increases with the rate of secretion. In the present study, however, no attempt was made to measure the rate of salivary flow.

No important association was found between caries experience and the various salivary constituents. These findings are in agreement with those previously reported for sodium,[13, 14] potassium,[15] and solids and ash.[11, 40-42]

SUMMARY

Whole, paraffin-stimulated saliva was collected from children aged 5 to 6 years (132 males, 106 females) and 12 to 14 years (188 males, 84 females). Least squares multiple regression equations were fitted relating the number of def or DMF teeth for each age and sex to the sodium and potassium concentrations, the log sodium-potassium ratio, and the per cent solids and ash. No significant relation was observed between the number of def or DMF teeth and the salivary constituents analyzed.

No significant sex differences were observed in the 5- to 6-year-old group, except for a slightly higher concentration of ash in the boys. In the 12- to

14-year-old group, the males showed a significantly higher sodium concentration, sodium potassium ratio, and per cent solids and ash than the females. No sex difference, however, was observed in the potassium concentration.

The sodium concentration, the sodium-potassium ratio and the per cent solids of the 12- to 14-year-old boys were significantly higher than those of the 5- to 6-year-old boys, whereas the potassium concentration was significantly lower. For girls, the only significant age change observed was an increase in the per cent solids.

The authors are indebted to Mrs. Virginia L. DuBois for her aid in the collection of the saliva specimens.

REFERENCES

1. Karshan, M.: Biochemical Aspects of Dental Caries, In Dental Science and Dental Art, S. M. Gordon, Philadelphia, 1938, Lea & Febiger, p. 327.
2. Dewar, M. R.: Laboratory Methods for Assessing Susceptibility to Dental Caries, *D. J. Australia* **21**: 509, 1951.
3. Roskin, M.: Mineral Content of Saliva of Children With Arrested Dental Caries, *Proc. Soc. Exper. Biol. & Med.* **25**: 465, 1928.
4. Hawkins, H. F.: A Rational Technique for the Control of Caries and Systemic Pyorrhea, *J. D. Res.* **11**: 201, 1931.
5. Karshan, M.: Factors in Saliva Correlated With Dental Caries, *J. D. Res.* **18**: 395, 1939.
6. Karshan, M., Rosebury, T., and Waugh, L. M.: Dental Caries Among Eskimos of the Kuskokwim Area of Alaska. II. Biochemical Characteristics of Stimulated Saliva Correlated With Dental Caries and Occurrence of Salivary Calculus, *Am. J. Dis. Child.* **57**: 1026, 1939.
7. Karshan, M., Siegel, E. H., and Waugh, L. M.: Biochemical Studies of the Saliva of Eskimos. Correlated With Dental Caries and the Occurrence of Salivary Calculus, *Am. J. Dis. Child.* **59**: 39, 1940.
8. Becks, H., Wainwright, W. W., and Young, D. H.: Does Salivary Calcium and Phosphorus Composition Differ Significantly in Caries-Free and Caries-Active Individuals? *J. D. Res.* **20**: 171, 1941.
9. Tóth, K.: Calcium, Phosphorus and Magnesium Content of Saliva of Caries Active and Caries Resistant Individuals, *Acta med. Acad. sc. hung.* **6**: 493, 1954.
10. Grove, C. J., and Grove, C. T.: Chemical Study of Human Saliva Indicating That Ammonia Is an Immunizing Factor in Dental Caries, *J. A. D. A.* **22**: 247, 1935.
11. Arnold, F. A., Jr., and McClure, F. J.: A Study of the Relationship of Oral *Lactobacillus acidophilus* and Saliva Chemistry to Dental Caries, *Pub. Health Rep.* **56**: 1495, 1941.
12. Ludwick, W. E., and Fosdick, L. S.: The Ammonia Content of the Mouth, *J. D. Res.* **29**: 38, 1950.
13. Knoche, E.: Ueber der Ionengehalt des Speichels and seine Beziehung zur Zahnkaries, *Deutsche Zahnarzt Wchnschr.* **30**: 419, 1927.
14. White, J., Bunting, R. W., Thomson, K., and Jay, P.: Relation Between Dental Caries and Potassium and Sodium Content of Saliva, *J. D. Res.* **16**: 315, 1937.
15. Wach, E. C.: Studies on the Potassium and Calcium Contents of Human Saliva, *J. D. Res.* **15**: 265, 1936.
16. Barnes, R. B., Richardson, D., Berry, J. W., and Hood, R. L.: Flame Photometry, *Indust. & Engin. Chem. (Indust. Ed.)* **17**: 605, 1945.
17. White, A. G., Gordon, H., and Leiter, J. L.: Studies in Edema. II. The Effect of Congestive Heart Failure on Saliva Electrolyte Concentrations, *J. Clin. Invest.* **29**: 1445, 1950.
18. Frawley, T. F., and Forsham, P. H.: The Salivary Na/K Ratio and Adrenal Salt Regulating Factors: Prolonged Salt Retention With Desoxycorticosterone Trimethyl Acetate, *J. Clin. Endocrinol.* **11**: 722, 1951.
19. Frawley, T. F., and Thorn, G. W.: The Relation of the Salivary Sodium-Potassium Ratio to Adrenal Cortical Activity, Proc. Second Clin. ACTH Conference **1**: 115, 1951 (Philadelphia, The Blakiston Co.).
20. Grad, B.: Influence of ACTH on Sodium and Potassium Content of Human Mixed Saliva, *Fed. Proc.* **10**: 53, 1951.
21. *Idem*: The Influence of ACTH on the Sodium and Potassium Concentration of Human Mixed Saliva, *J. Clin. Endocrinol.* **12**: 708, 1952.

22. Dreizen, S., Niedermeier, W., Reid, A. I., and Spies, T. D.: The Effect of ACTH and Cortisone on the Sodium and Potassium Levels of Human Saliva, *J. D. Res.* **31**: 271, 1952.
23. Pawan, G. L. S.: Studies on the Salivary Sodium/Potassium Ratio in Man, *Biochem. J.* **60**: xii, 1955.
24. Bowman, R. L., and Berliner, R. W.: Principles of Design and Operation of Internal Standard Flame Photometers for Sodium and Potassium Determination, *Fed. Proc.* **8**: 14, 1949.
25. Everett, M. R.: Medical Biochemistry, ed. 2, New York, 1946, Paul Hoeber, Inc., p. 582.
26. Hawk, P. B., Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, ed. 13, New York, 1954, The Blakiston Co., Inc., p. 350-351.
27. Clark, G. W.: The Influence of Diet Upon the Inorganic Constituents of Human Saliva, *D. Cosmos* **69**: 500, 605, 1927.
28. Woldring, M. G.: Vlamfotometrische Bepaling Van Natrium en Kalium in Enkele Biologische Vloeistoffen, Drukkerij I., Oppenheim N. V.—1952, Groningen, Thesis.
29. Muhler, J. C., and Swenson, H. M.: Preparation of Synthetic Saliva From Direct Analysis of Human Saliva, *J. D. Res.* **26**: 474, 1947.
30. Mathis, H.: Beitrage zur chemie des Spechels. III. Ueber den Gehalt des gemischten Mundspeichels an der Kationen: Kalium, Kalzium and Natrium sowie an der anionen Chlor und Phosphor, *Ztschr. f. Stomatol.* **32**: 1188, 1248, 1934.
31. Dreizen, S., Reid, A. I., Niedermeier, W., and Spies, T. D.: Sodium and Potassium as Constituents of Human Salivary Buffers, *J. D. Res.* **32**: 497, 1953.
32. Bernstein, R. E.: The Determination of Potassium and Sodium in Biological Fluids by Flame Spectrophotometry, *South African J. M. Sc.* **17**: 101, 1952.
33. Woldring, M. G.: Vlamfotometrische Bepaling van natrium en kalium in enkels biologische Vloestoffen, *Pharmaceutisch Weekblad.* **87**: 725, 1952.
34. Niedermeier, W., Dreizen, S., Stone, R. E., and Spies, T. D.: Sodium and Potassium Concentrations in the Saliva of Normotensive and Hypertensive Subjects, *Oral Surg., Oral Med., & Oral Path.* **9**: 426, 1956.
35. Niedermeier, W.: Age as a Factor in Sodium and Potassium Concentrations of Stimulated and Unstimulated Mixed Human Saliva, *Fed. Proc.* **12**: 251, 1953.
36. Becks, H.: Ergebnisse neuerer Speichel-Untersuchungen, Berlin, 1929, H. Meusser.
37. Grad, B.: Diurnal and Age Changes in the Sodium and Potassium Concentration of Human Mixed Saliva, *J. Gerontol.* **6**: 93, 1951.
38. Hungerland, H., Qwenzlein, I., and Weber, H.: Über Das Verhalten der Natrium and kalium-Konzentration des Speichels in Sauglings-und Kindesalter, *Klin. Wochschr.* **33**: 44, 1955.
39. Brown, J. B., and Klotz, N. J.: Studies on the Chemistry of Mixed Human Saliva. II. Sodium, Potassium, and Calcium in Salivas Secreted at Widely Varying Rates, *J. D. Res.* **16**: 19, 1937.
40. Lothrop, A. P., and Gies, W. J.: A Chemical Study of Saliva in Its Probable Relation to the Decay of Teeth, *J. Allied Dent. Soc.* **5**: 262, 1910.
41. Hubbell, R. B.: The Chemical Composition of Saliva and Blood Serum of Children in Relation to Dental Caries, *Am. J. Physiol.* **105**: 436, 1933.
42. White, J., and Bunting, R. W.: A Comparison of the Chemical Composition of Stimulated and Resting Saliva of Caries-Free and Caries-Susceptible Children, *Am. J. Physiol.* **117**: 529, 1936.

EARLY ADMINISTRATION OF VARIOUS CARBOHYDRATES AND SUBSEQUENT DENTAL CARIES IN THE RAT

R. R. STEINMAN AND M. I. HALEY

College of Medical Evangelists, School of Dentistry, Loma Linda, Calif.

CARBOHYDRATES, usually in the form of lactose, are a normal constituent of milk. Sognnaes,[1] from a study of rats which were kept on a high sucrose diet through several generations, concluded that the ingestion of sucrose during the formative period of the teeth affects the subsequent caries experience of these teeth due to a change in the maternal milk or due to the high sucrose diet eaten ad libitum after the eyes opened. The teeth of Rhesus monkeys formed during the period of a high sucrose diet also were susceptible to decay, whereas those formed before the animals were placed on a high sucrose diet were immune. An analysis of the caries experience of Scandinavian children during and after World War II tend to support this conclusion.[2] The addition of ashed laboratory chow to the refined rations fed dams caused a decrease in the amount of decay produced in the offspring. This might suggest that the increase of caries on the refined diet alone was due to a lack of some important unknown constituent in the diet.[3]

The period of development of the molars of the white rat from the beginning of calcification to eruption into the oral cavity has been shown by Hoffman and Schour.[4] This period of developmental time for the first and second molars roughly corresponds to the suckling period. The calcification of the third molars involves only the last quarter of the suckling period and extends approximately 14 days beyond this date. To determine the possible effects that various carbohydrates may have on the molars from the beginning of calcification to their eruption into the mouth, this study was conducted.

EXPERIMENTAL

Stock animals obtained from the Naval Dental Research Institute, Bethesda, Maryland, were known to be caries susceptible on the Hopper, Webber, Canniff diet. These animals were also found to be caries susceptible on a high sucrose diet.

All animals, after weaning, were placed on a high sucrose diet (Table I).

The following solutions were administered to 8 groups of young rats during the suckling period by means of a calibrated eye dropper, 20 per cent sucrose, 20 per cent lactose, 20 per cent raw cornstarch suspension, 20 per cent glucose, 20 per cent equal parts of glucose and fructose, and tap water.

Received for publication April 15, 1956; revised by author April 4, 1957.
Presented at the 34th General Meeting of the International Association for Dental Research, St. Louis, Mo., March 22-25, 1956.

TABLE I
HIGH SUCROSE DIET

Casein	24
Sucrose	65
Salts	4
Corn oil	5
Dehydrated whole liver	4
Vitamin	2
Choline	0.2

Note: Vitamins A and D drops were given once a week.

The drops were given to the young, morning, noon, and evening each day, until the animals were weaned. The young were allowed to nurse ad libitum. The amount of carbohydrates given depended upon the age and weight of the animals. The ratio of carbohydrate to body weight up to the fifth day was 1 to 1,000, and by the sixth day the ratio was increased to about 1 to 750. The average weights at the time of weaning showed no significant difference between groups with the exception of the animals that received the starch suspension. The animals that received the starch suspension were 16 per cent lighter in weight than their littermates which received the 20 per cent lactose solution.

Between 2 and 3 days after birth, the young from 2 litters were exchanged, one half going to each dam. The dams did not object to the trade. One group of 6 animals received the 20 per cent sucrose solution 3 times a day and the other group of 9 animals received an equal amount of water, as a control, 3 times a day until weaned. The animals were weaned at 21 days of age and placed on identical high sucrose diets (Table I). The rats were sacrificed after 14 weeks and the molars were examined by grinding successive planes parallel to the occlusal surface. Individual charts which showed the relationship of the enamel, dentin, and the pulp of each molar were used to record the caries. The results are shown in Table II.

TABLE II
CARIOUS LESIONS IN ANIMALS RECEIVING SUCROSE SOLUTION COMPOUND AND WATER

	NUMBER OF ANIMALS	FIRST MOLAR	SECOND MOLAR	THIRD MOLAR	TOTAL
20% sucrose	6	17.3	10.4	4.9	32.8
Water	9	6.3	5.4	1.1	12.8
T value		8.0	3.2	4.0	

Two additional litters were exchanged the third day after birth. One group of 7 animals was given 20 per cent lactose, and the other group of 8 animals received a 20 per cent raw cornstarch suspension 3 times a day, morning, noon, and night. When weaned, all rats were placed on the high sucrose diet (Table I) for 14 weeks. The results obtained from this study are shown in Table III.

TABLE III
DENTAL CARIES IN ANIMALS RECEIVING LACTOSE DROPS AND STARCH

	NUMBER OF ANIMALS	FIRST MOLAR	SECOND MOLAR	THIRD MOLAR	TOTAL
20% lactose	7	4.5	2.7	0.9	8.1
20% raw starch	8	24.6	17.1	10.9	52.6
T value		3.6	2.7	3.3	

The number of cavities found in each group was quite uniform with the exception of the group given starch. Some animals in the starch group had caries-free teeth while others had teeth decayed off even with the gingival margin. The animals with high caries counts were the ones which weighed the least at weaning.

In a further study of this problem, each litter from 7 dams was divided into 4 groups of approximately 15 animals each. Starting the day after birth, the following solutions were given to the young rats three times a day, morning, noon, and night until they were weaned at 21 days of age: 20 per cent sucrose, 20 per cent lactose, 20 per cent glucose, and 20 per cent mixture of equal parts of glucose and fructose.

All animals were placed on the high sucrose diet (Table I) at weaning for a period of 13 weeks.

Seventy-two animals were born, 65 survived until weaning, and 62 survived to the end of the study. A summation of this part of the study is given in Table IV.

TABLE IV

CARIOUS LESIONS IN ANIMALS ON VARIOUS CARBOHYDRATES STARTED 24 HOURS AFTER BIRTH

SOLUTION	NUMBER OF ANIMALS	FIRST MOLAR	SECOND MOLAR	THIRD MOLAR	TOTAL
20% sucrose	14	26.4	20.1	6.1	52.6–
20% lactose	15	18.4	13.2	3.1	34.7
20% glucose	18	20.3	13.8	2.8	36.9
20% glucose and fructose	15	22.0	14.4	4.8	41.2

T values—Sucrose-lactose 2.45
Sucrose-glucose and fructose 1.76
Sucrose-glucose 2.96

Starting the administration of the carbohydrate solutions 48 hours earlier greatly increased the amount of caries. The first and second molars were quite uniformly discolored. The second molar was usually a darker yellow than the first molar. Even when the first and second molars were decayed to the gingival margin, the third molar was left intact and was a normal white color. The number of cavities found in each group was fairly uniform with the exception of the group receiving the lactose. Some in this group had practically no caries, while their littermates had molars decayed to the gingival margin.

DISCUSSION

It was felt that the quantities of carbohydrates used in these studies were not excessive. There were no differences in the weaning weights between groups, with the exception of the group which received the raw starch suspension. These were found to weigh 16 per cent less than their littermates which received the 20 per cent lactose solution.

All the young rats were on a normal maternal milk diet with a small amount of carbohydrate added. The small amount of carbohydrate appeared to cause quite profound changes in the teeth. No effort was made to change the quality or quantity of the maternal milk in these studies. This study would suggest

that the formula of the milk may be of considerable importance, not only as to the amount of carbohydrate, but the kind used. The low number of deaths from birth to weaning would also indicate that the amount of carbohydrate administered was not excessive.

In comparing the results shown in Tables II and III with those in Table IV, the importance of the early administration of the carbohydrates is shown. Although these animals were not littermates, and a ertical comparison cannot be made, they were from the same dams. However, it should be obvious that the animals that received the carbohydrate solutions the day after birth had a higher caries score. Since changes were produced in the third molars of littermates on different carbohydrates, this points to the importance of the early period of tooth development as a time during which structural imperfections may develop. The earlier the drops were started, the greater the number of teeth that were found to be totally destroyed. Sucrose consistently affected the later caries experience of the teeth to a greater extent than did the other carbohydrates.

CONCLUSIONS

1. The administration of various carbohydrates from the beginning of calcification of the molars to their eruption into the oral cavity was shown to affect the later caries experience of these teeth.

2. Carbohydrates differ in their ability to produce this change.

3. This period appears to occur during the early part of the calcification of the teeth.

Grateful acknowledgment is made to Mr. R. D. Walters for his technical assistance throughout this study.

REFERENCES

1. Sognnaes, R. F.: Caries in Rodents on a Purified Diet, *J. A. D. A.* **37**: 676, 1948.
2. *Idem:* An Analysis of a War-time Reduction of Dental Caries in European Children With Special Regards to Observations From Norway, *Am. J. Dis. Child.* **75**: 792, 1948.
3. *Idem:* Experimental Rat Caries. IV. Effect of Natural Salt Mixture on the Caries-conduciveness of an Otherwise Purified Ration, *J. Nutrition* **53**: 195, 1954.
4. Hoffman, M. M., and Schour, I.: Quantitative Studies in the Development of the Rat Molar, *Anat. Rec.* **78**: 233, 1940.

INCIDENCE OF CARIES AT DIFFERENT LOCATIONS ON THE TEETH

JOHN H. BARR, D.D.S., B.Sc.DENT., RONALD R. DIODATI, M.S., AND RUSSELL G. STEPHENS, D.D.S.

Division of Oral Diagnosis, Tufts University School of Dental Medicine, Boston, Mass.

THE published literature indicates little concern about information on the distribution of carious attacks further than counts of the average number of teeth or surfaces per mouth affected by caries; and there is already evidence to suggest that neither of these measures may have more than limited value.

From the studies which have been made of the distribution of the caries attack on teeth by specific types, locations, or surfaces, only a very incomplete picture may be glimpsed. It is surprising, in the face of the tremendous effort that has been made to learn the nature of the carious process through the past 60 years, that this aspect of the problem has apparently not attracted closer attention.

All the earlier studies of the kind indicated have been characterized by various limitations, though collectively they may be viewed as offering a valuable nucleus of information. Arnim[3] analyzed data from several independent surveys into a kind of rating, by tooth surfaces affected; limited in their value, however, by the absence of radiographic findings for proximal attacks, and by an arbitrary grouping of attack frequencies into 5 categories. One of the present authors[5] has presented more detailed data on proximal attacks, but it was limited to median age levels of from 7.5 to 22.5 years, and did not report the pit or fissure or the cervical attacks. More recently, Dirks, Winkler, and Van Aken[14] and Galagan and Vermillion,[17] intending primarily to study the radiographic technic for examination of proximal surfaces, have presented data confined to proximal surfaces of posterior teeth in adolescents. These studies, even collectively, offer neither a sufficiently comprehensive range nor are they on a properly comparable basis so as to permit the compilation of figures to indicate the pattern of incidence of caries by location or type for each tooth of the dentition.

DESIGN OF STUDY

Opportunity was taken to add to a comprehensive program, planned for study of the epidemiology of periodontal disease, sufficient additional examinations to provide data on the distribution of carious attacks on the teeth. The

Presented at the Thirty-fourth General Meeting of the International Association of Dental Research, St. Louis, Mo., March 24, 1956.

This study was supported in part by the Student Research Fellowship program of the U. S. Public Health Service. Certain clinical examinations were made through the courtesy of C. D. Marshall-Day and L. F. Quigley as part of an investigation carried out under Contract No. D.A.49-007-MD-188, Office of the Surgeon General, Dept. of the Army. Ronald E. Shor, M.S., and Donald Giddon, M.S., prepared the statistical evaluation of percentage differences.

Received for publication June 16, 1956.

additional examinations were designed to study the distribution and interrelations of what were viewed as differing types of attack,[7, 18] namely, pit or fissure caries and each of 2 forms of smooth surface lesions (proximal and cervical). This approach was selected because it was felt it would best contribute to further understanding of the nature of the caries attack and was well supported by clinical impressions as to the possible absence of correlation between the 3 types mentioned. It might be felt desirable to have had even more detailed data on the locations at which each type of lesion is found, but it was considered more important to study which teeth suffered which kinds of attack than the actual number of separate attacks occurring on each tooth. However, findings for proximal caries experience of mesial and distal surfaces were recorded separately to permit study of known predisposing factors such as approximation or spacing of teeth.

Five hundred and eighty-three patients (256 ♂, 327 ♀), ranging from 13 to 40 years of age were examined. It was felt that, although patients above 40 years of age were available and could have been included, findings beyond the 40-year level would tend to be unreliable by reason of a sharp increase in the number of missing teeth and in the number of exposed root sufaces, the former confusing the data for all types of caries and the latter favoring a disproportionately high incidence of cervical attack.

Careful clinical examination for pit or fissure and for cervical lesions was made by one examiner throughout each age group. Radiographic examination was relied on for proximal caries experience, findings being reported by one examiner for all age groups. It is readily admitted that the addition of a clinical examination of proximal surfaces may have been desirable, but in view of the already exhaustive medical and oral examinations programmed for patients in the survey, it was quite impossible to arrange for this. The recent report by Galagan and Vermillion[17] has been noted with interest, but the variability of the radiographic reporting transpires, it should be noted, as being sharply less than that found for clinical examination of proximal surfaces.[4] The remarkable efficiency of radiographic examination for proximal surfaces has been demonstrated.[4, 6, 8, 22] What appear to be less convincing measures in other studies[11, 12, 16] are figures for percentages of total cavities or of DMF teeth and, it may be assumed, if applied to the proximal surfaces only, that these studies would fully support the premise that radiographic examination is adequate and consistent for our present purpose. Data to be presented for proximal caries experience will be found to correspond closely with previously published findings based on both clinical and radiographic examination.[5, 22]

PROCESSING OF DATA

In the processing of data, crowned or otherwise completely restored teeth have been included with missing teeth (Table I) because no information is available on the type or types of caries attack responsible for their restoration. The term "surviving teeth" should therefore be construed to exclude both missing and completely restored teeth.

TABLE I

UPPER TEETH	13-20 YR. 504 QUADRANTS (A)*	(B)†	21-30 YR. 310 QUADRANTS (A)	(B)	31-40 YR. 352 QUADRANTS (A)	(B)	LOWER TEETH	13-20 YR. 504 QUADRANTS (A)	(B)	21-30 YR. 310 QUADRANTS (A)	(B)	31-40 YR. 352 QUADRANTS (A)	(B)
1	8		10		48		1	4		11		12	
2	4		24		50		2	2		6		6	
3	9		16		33		3	0		5		6	
4	10	123	25	158	64	180	4	10	40	13	75	25	92
5	9	137	43	151	79	188	5	19	116	43	140	58	145
6	25	211	64	148	108	158	6	79	230	125	128	163	131
7	32	45	21	91	57	103	7	30	75	41	111	59	127

†(b) Teeth with occlusal extensions of proximal fillings.
*(a) Number of missing or completely restored teeth.

The data from examination charts were transferred to IBM cards, coded to receive the following information for each of 28 teeth (third molars not included).

1. Mesial caries
2. Distal caries
3. P and F caries
4. Cervical caries
5. Mesial filling
6. Distal filling
7. P and F filling
8. Cervical filling
9. Tooth completely restored
10. Tooth missing
11. Spacing mesial to tooth
12. Spacing distal to tooth

In addition, information was entered on the IBM cards covering age, sex, and number of teeth missing in each quadrant, in each arch, and in each mouth.

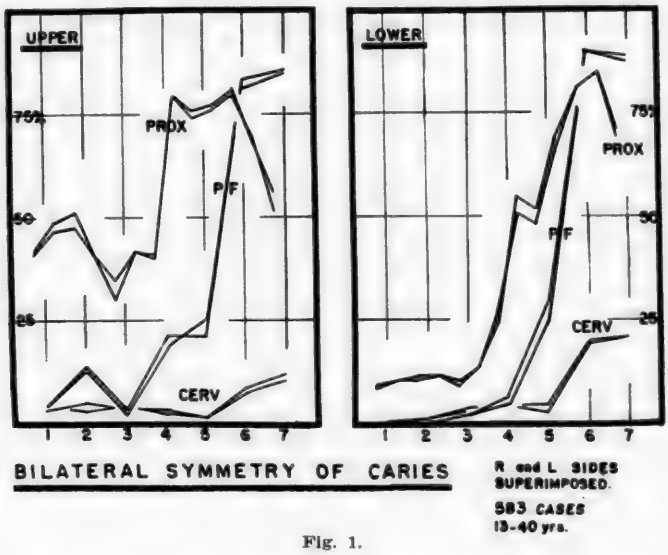


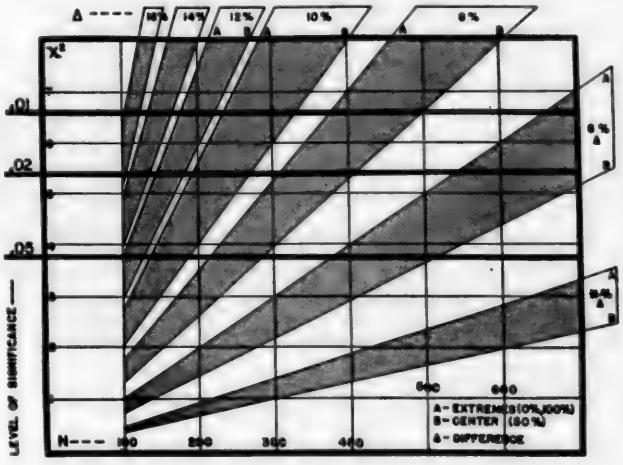
Fig. 1.

The data were examined for bilateral symmetry of the pattern of grouped findings for each type of caries experience in surviving teeth (Fig. 1). The close correspondence of the right and left sides shown in these graphs, supported by earlier studies,[3, 5] is believed to justify the pooling of data from right and left sides of the mouth for purposes of all further analysis.

At this point it is advisable to comment on the basis on which all further analyses have been presented. There has been little support for the previous suggestion of one of the authors[5] that in many cases where teeth are missing, reference could be made to the opposite side of the mouth, though Grainger and Reid[18] have since discussed the procedure. Data have therefore been presented in 2 widely used forms, (1) as a percentage of surviving teeth on both sides (DFS teeth, i.e., decayed, filled, or sound teeth), and (2) as a percentage of the original number of teeth of both sides (DFSM—decayed, filled, sound, or missing). This may facilitate comparison with other data which may become available; it will certainly prove to dramatize a wide discrepancy between results obtained by these methods of computation, especially for pit or fissure and for cervical types of attack on certain teeth. It underscores doubts which have arisen[10, 18, 21] concerning the arbitrary custom of assuming all 5 surfaces[20] of each missing tooth as having been attacked by caries.

STATISTICAL EVALUATION OF PERCENTAGE DIFFERENCES

Fig. 2 shows the approximate level of significance for any percentage differences in the findings to be presented—percentage differences between the incidence of caries on individual teeth of the dentition, between different age levels for any given tooth, or between the 2 methods of computation used for the



SIGNIFICANCE OF DIFFERENCES BETWEEN PERCENTAGES.

Fig. 2.

caries experience of any tooth. The chart is based on the chi-square test for the difference between 2 uncorrelated proportions. In this statistic the assumption is made that no correlation exists between the percentages being compared, and it therefore results in a conservative estimate of the significance of the differences. Chart readings have been confirmed by comparisons with exactly computed chi-square levels. In using the chart to compare findings for groups of unequal size, the average N (number) for the 2 groups should be used.

It may be useful to summarize the evaluation of percentage differences in relation to the findings which follow. Where N is 300, differences of 8 per cent fall within the 0.05 level of confidence, differences of 10 per cent within the 0.02 level, and differences of 12 per cent or more fall within the 0.01 level of significance. Where N exceeds 500, differences of 6 per cent fall within the 0.05 level and differences of 8 per cent or more fall within the 0.01 level of significance.

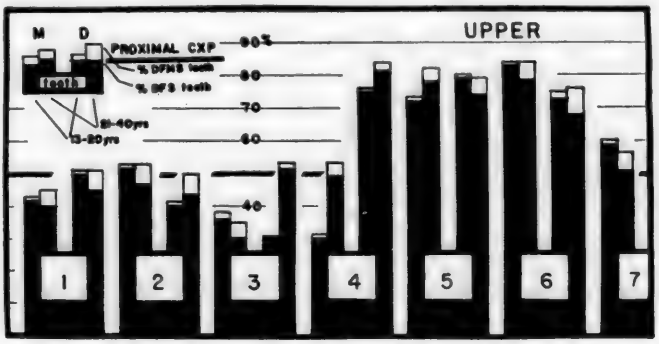


Fig. 3.

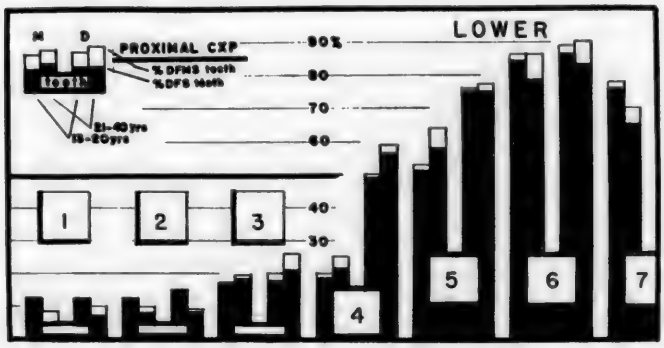


Fig. 4.

FINDINGS AND COMMENT

(a) *Proximal Caries Experience.*—Table II and Figs. 3 and 4 show the percentage incidence of caries on mesial and distal surfaces of individual teeth according to age groups, both on the basis of surviving (DFS) teeth and the original number (DFSM) of teeth. Notable observations are the relative frequency with which specific surfaces are affected; the negligible increment of attack after the twenty-first year, excepting for the distal surface of the upper canine and the approximating mesial surface of the upper first premolar; and generally unimportant variations between the alternative bases of computation of percentage incidence. Increased numbers of missing teeth in the upper age group tend in numerous instances to compensate closely for a fall in incidence

on the basis of surviving teeth. It may be inferred that many of the missing teeth have been lost solely by reason of proximal caries attacks, if it is found that other types of attack are much less frequent on the teeth surviving for study. The drop in incidence based on surviving teeth is not likely to have arisen from drifting and spacing after the limit of the lower age group, for it is apparent that, except for the specific instances noted above, there is no important later increment in proximal caries experience.

TABLE II

PROXIMAL CARIES EXPERIENCE

UPPER TOOTH AND SURFACE	13-20 YEARS 504 QUADRANTS		21-40 YEARS 662 QUADRANTS		LOWER TOOTH AND SURFACE	13-20 YEARS 504 QUADRANTS		21-40 YEARS 662 QUADRANTS	
	(A)*	(B)†	(A)	(B)		(A)	(B)	(A)	(B)
1 mesial	42.7	43.7	39.7	45.0	1 mesial	12.6	13.3	5.3	8.6
1 distal	50.0	50.7	45.8	50.6	1 distal	12.4	13.1	7.2	10.4
2 mesial	52.0	52.5	47.1	53.0	2 mesial	12.7	13.1	7.5	9.2
2 distal	39.8	42.4	44.0	50.3	2 distal	14.7	15.1	7.7	9.4
3 mesial	35.8	37.9	30.1	35.4	3 mesial	11.9	11.9	6.3	7.9
3 distal	30.1	31.4	50.8	54.6	3 distal	16.5	16.5	18.1	18.7
4 mesial	29.8	31.2	49.7	56.5	4 mesial	18.2	20.2	21.5	26.0
4 distal	75.2	75.7	81.7	84.4	4 distal	49.0	50.0	56.6	59.0
5 mesial	73.2	73.7	78.3	82.3	5 mesial	50.7	53.0	58 1	64.4
5 distal	79.1	80.3	74 0	78.7	5 distal	75.2	76.1	74.8	78.7
6 mesial	83.7	84.4	78.2	83.8	6 mesial	83.6	86.2	78.2	87.7
6 distal	73.2	74.5	67.1	75.6	6 distal	87.2	89.4	82.8	90.2
7 mesial	57.8	60.5	49.7	55.6	7 mesial	76.4	77.7	65.0	70.2

*(a) Carious plus restored surfaces (DF), as a percentage of surviving teeth (DFS).
†(b) Carious, restored, and missing surfaces (DFM), as a percentage of the original number of teeth (DFMS).

(b) *Pit or Fissure Caries Experience.*—In Table III and Fig. 5, showing the percentage incidence of pit or fissure caries experience for individual teeth by age groups, posterior teeth with proximal restorations have been eliminated in computations on the surviving teeth basis as there is no means of knowing the condition of the occlusal pit or fissure at the time the proximal restoration was placed with its customary extension over to the occlusal surface. The posterior teeth with proximal restorations have, of course, been included in the alternative DFMS teeth computation, and the chart gives a strong indication that, for the premolars, the assumption of occlusal caries experience would often be in error in cases where occlusal surfaces have been restored as part of a proximal restoration with occlusal dovetail.[9] The customary assumption of pit or fissure caries experience in the case of any missing teeth anterior to the molars is equally difficult to justify for purposes of counts of DMF surfaces. As in the case of proximal lesions, the relative incidence of caries experience in pits or fissures is clearly shown for individual teeth, and there is no important increment after the twenty-first year for any of the teeth.

TABLE III

PIT OR FISSURE CARIES EXPERIENCE

UPPER TOOTH NUMBER	13-20 YEARS 504 QUADRANTS		21-40 YEARS 662 QUADRANTS		LOWER TOOTH NUMBER	13-20 YEARS 504 QUADRANTS		21-40 YEARS 662 QUADRANTS	
	(A)*	(B)†	(A)	(B)		(A)	(B)	(A)	(B)
1	3.8	5.4	4.6	13.0	1	--	1.0	--	3.9
2	12.4	13.1	13.9	23.6	2	--	0.8	--	2.4
3	3.2	5.0	3.4	10.6	3	1.8	1.8	1.8	3.5
4	22.4	43.0	18.7	71.2	4	9.9	18.8	11.1	38.7
5	22.0	44.6	25.5	77.3	5	26.4	40.8	25.7	69.0
6	82.0	90.5	81.5	94.8	6	90.7	96.0	87.0	97.9
7	67.0	72.3	88.5	92.5	7	88.0	90.4	88.0	94.4

*(a) Teeth with carious or restored pits or fissures (DF), as a percentage of surviving teeth (DFS), excluding teeth with occlusal extensions of proximal fillings.

†(b) Teeth with carious or restored pits or fissures (including extensions of proximal fillings), plus missing teeth (DFM), as a percentage of the original number of teeth (DFMS).

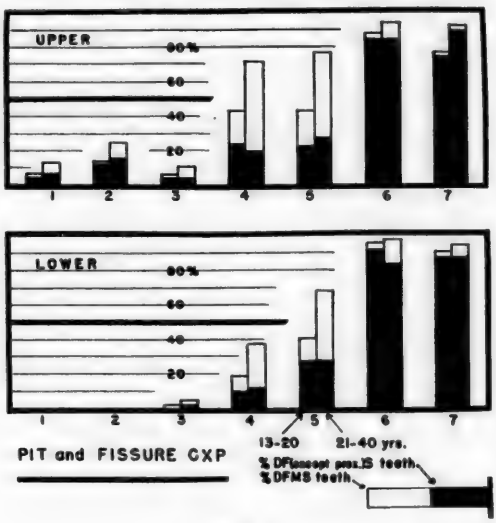


Fig. 5.

(c) *Cervical Caries Experience.*—Table IV and Fig. 6 show the percentage incidence of cervical caries attack for individual teeth in similar form to the preceding tables and diagrams, but with a more detailed breakdown of the age groups beyond the twenty-first year because of the continuing increment which appeared through the upper age levels.[15] There is, on the basis of surviving teeth, a generally progressive increase in the frequency of cervical attack through the three decades, excepting in the case of lower molar teeth. The relative frequency of attack for different individual teeth is self-evident from the chart, and an apparent oddity is the extremely low incidence of cervical caries on the upper second bicuspid. This corresponds with a previous observation by Arnim.[3] Again there are conspicuous indications of the effect of any assumption that all 5 surfaces of missing teeth should be regarded as having been carious.

TABLE IV

CERVICAL CARIES EXPERIENCE

UPPER TOOTH	13-20 YEARS 504 QUADRANTS (A)*	(B)†	21-30 YEARS 310 QUADRANTS (A)	(B)	31-40 YEARS 352 QUADRANTS (A)	(B)	LOWER TOOTH	13-20 YEARS 504 QUADRANTS (A)	(B)	21-30 YEARS 310 QUADRANTS (A)	(B)	31-40 YEARS 352 QUADRANTS (A)	(B)
1	0.8	2.4	3.3	6.5	7.9	20.4	1	--	0.8	0.7	4.2	0.3	3.7
2	2.4	3.2	3.7	11.0	5.6	19.0	2	--	0.4	1.6	3.5	1.2	2.8
3	1.1	2.8	4.1	9.0	10.6	19.0	3	1.4	1.4	7.9	9.4	8.7	10.4
4	0.6	2.6	1.8	9.7	6.3	23.2	4	2.8	4.8	10.4	14.2	16.5	22.4
5	0.4	2.2	0.8	14.5	2.9	24.6	5	3.1	6.8	7.5	20.2	15.6	29.0
6	6.8	11.3	7.7	27.0	10.2	37.8	6	20.4	33.0	17.3	50.1	26.0	60.5
7	8.9	14.7	9.3	15.5	12.5	26.6	7	19.4	24.0	20.0	30.6	25.0	37.5

*(a) Teeth with cervical caries or fillings (DF) as a percentage of surviving teeth (DFS).

†(b) Teeth with cervical caries or fillings, plus missing teeth (DFM), as a percentage of the original number of teeth (DFMS).

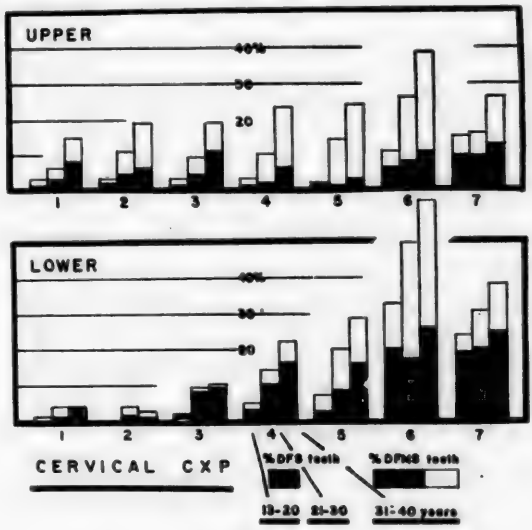


Fig. 6.

(d) *Correlation Between Frequency of Proximal and Cervical Attacks.*—Study of the charted data, for correlation between the incidence of the 2 forms of smooth-surface caries, supports a clinical impression that certain types of carious attack may be quite unrelated. Scatter-diagrams (Figs. 7 and 8) were prepared, plotting for each mouth the number of proximal attacks against the number of teeth with cervical attacks.

Lines of regression have been drawn, based on inspection of frequency means, and there is no need of further statistical analysis to show the absence of any correlation of predictive value between the incidence of proximal and cervical caries in individuals. The data did not readily permit a comparable correlation between the frequency of pit or fissure attacks with these 2 types, but it is hoped that this may become possible at a later date; we have the impression that significant correlation between these types is equally unlikely.

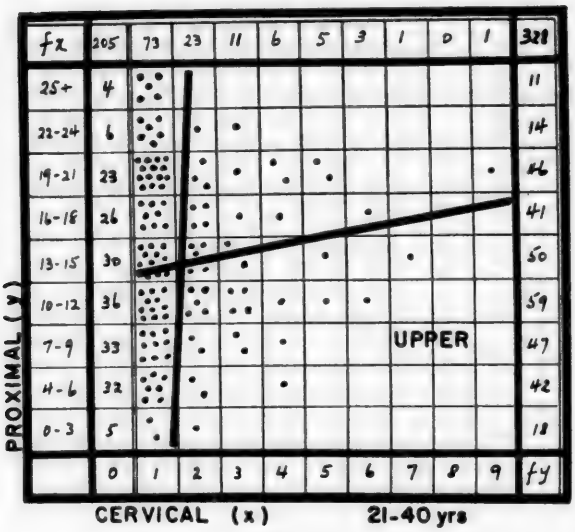


Fig. 7.

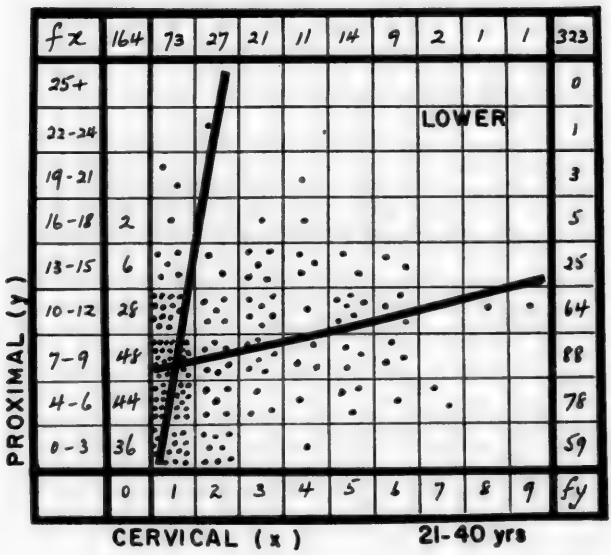


Fig. 8.

DISCUSSION AND SUMMARY

The percentage incidence of 3 types of carious attack—pit or fissure, proximal, and cervical—has been reported for all permanent teeth excepting the third molars for age groups ranging from 13 through 40 years. The data presented should prove useful as a background for caries diagnosis and prognosis and may contribute to understanding of the caries process. The pattern of attack indicates in general little increment in pit or fissure or in proximal caries experience

after 21 years of age, but a progressive cervical attack throughout the age groups studied. No correlation was found between the incidence of the 2 forms of smooth-surface caries—proximal and cervical. There was wide divergence in findings for both pit or fissure and for cervical types of lesions when computed with or without assumptions for caries in missing or restored teeth. Studies designed to evaluate potential caries-inhibiting agents should be designed to analyze the effectiveness separately for each type of carious attack and should not include arbitrary assumptions to cover missing teeth. Extensive studies, preferably of the vertical or continuing type, are needed to obtain further information on incremental patterns of carious attacks. This information could have predictive value useful to both clinical researchers and to those attempting the formulation of plans for group dental care.

REFERENCES

1. American Dental Association: Clinical Testing of Dental Caries Preventives, 1955.
2. Arnim, S. S.: Utilization of Biologic Principles in the Practice of Operative Dentistry, *J. A. D. A.* **28**: 598, 1941.
3. *Idem:* Utilization of Biologic Principles in the Practice of Operative Dentistry, *J. D. Res.* **21**: 243, 1942.
4. Barr, J. H.: The Diagnostic Value of Radiographic Examinations for Proximal Caries in Deciduous Posterior Teeth, *New Zealand D. J.* **41**: 89, 1945.
5. *Idem:* Some Characteristics of Caries on the Proximal Surfaces of the Teeth, *J. D. Res.* **28**: 466, 1949.
6. Barr, J. H., and Gresham, A. H.: The Detection of Carious Lesions on the Proximal Surfaces of Teeth, *J. A. D. A.* **41**: 198, 1950.
7. Baume, L. J.: Requirements for Recording Data of Dental Examinations in Caries Controls Studies, *D. Pract.* **5**: 376, 1955 (per *Year Book of Dentistry*, 1955-56, p. 95).
8. Blayney, J. R., and Greco, J. F.: The Value of Roentgenological vs. Clinical Procedures for the Recognition of Early Carious Lesions on Proximal Surfaces of Teeth, *J. D. Res.* **31**: 341, 1952.
9. Boyd, J. D., Cannon, J. J., and Leighton, R. E.: Epidemiologic Studies in Dental Caries. V. Placement of Fillings as a Source of Statistical Error in Estimates as to Dental Caries Progression Rates, *J. D. Res.* **31**: 354, 1952.
10. Bruckner, R. J., Hill, T. J., and Wollpert, B. J.: Measurement of Dental Caries Incidence in School Children Using a Sodium Bicarbonate Dentifrice, *J. D. Res.* **31**: 105, 1952.
11. Cheyne, V. D., and Horne, E. V.: The Value of the Roentgenograph in the Detection of Carious Lesions, *J. D. Res.* **27**: 58, 1948.
12. Chilton, N. W., and Greeneald, L. E.: Studies in Dental Public Health Administration. 11. Role of Roentgenograms in Public Health Dental Surveys, *J. D. Res.* **26**: 129, 1947.
13. Dirks, O. B., Van Amerongen, J., and Winkler, K. C.: A Reproducible Method for Caries Evaluations, *J. D. Res.* **30**: 346, 1951.
14. Dirks, O. B., Winkler, K. C., and Van Aken, J.: A Reproducible Method for Caries Evaluation, *J. D. Res.* **32**: 18, 1953.
15. Dunning, J. M.: A Comparison of Dental Caries on the Buccal and Proximal Surfaces of Premolar Teeth, *J. D. Res.* **20**: 195, 1941.
16. Dunning, J. M., and DeWilde, H.: Variations in the Efficiency of Bitewing Roentgenograms as Related to Age of Patients, *J. A. D. A.* **52**: 138, 1956.
17. Galagan, D. J., and Vermillion, J.: Diagnosis of Caries by Radiographic Interpretations, *J. D. Res.* **35**: 33, 1956.
18. Grainger, R. M., and Reid, D. B. W.: Distribution of Dental Caries in Children, *J. D. Res.* **33**: 613, 1954.
19. Gruebbel, A. D.: Caries Control—Public Health Aspects, *J. A. D. A.* **37**: 426, 1948.
20. Klein, H., and Palmer, C. E.: Studies on Dental Caries: Prevalence and Incidence of Dental Caries Experience, Dental Care and Carious Defects Requiring Treatment in High School Children, *Pub. Health Rep.* **55**: 1258, 1940.
21. McLaren, H. R.: A Study of Dental Caries Experience in Children of School Age, *J. C. D. A.* **14**: 466, 1948.
22. Porter, K. O., and Woods, E.: Dental Caries Prevalence in Children 15 and 16 Years of Age in Three Idaho Communities, *J. D. Res.* **33**: 542, 1954.

DENTAL CARIES AND DENTAL CARE NEEDS IN BALTIMORE SCHOOL CHILDREN (1955)

H. BERTON McCAULEY, D.D.S., AND TODD M. FRAZIER, A.B.

Bureau of Dental Care and Bureau of Biostatistics, Baltimore City Health Department, Baltimore, Md.

THE prevention of dental disease in a population requires repeated study of the epidemiology of tooth decay as it occurs in that population. Accordingly, two dental surveys have been conducted recently by the Baltimore City Health Department among the city's school children. The first, in March, 1952, yielded broad estimates of tooth decay experience in 12,000 public and parochial school children, aged 5 through 18 years.[1] A second, conducted between March 15 and May 19, 1955, was designed to test the validity of the 1952 findings and to inquire further into the relative importance of biosocial determinants of dental caries, such as sex, race and socioeconomic status, and the preventive role of oral hygiene.

The 1955 survey was concerned with a much smaller and more selective group of children than the earlier one. The later study included 2,520 public school pupils, 6, 8, and 10 years old, equally divided by age and sex. Although fluoridation of the Baltimore City water supply was begun Nov. 26, 1952, 27 months before the survey, there was no reason to anticipate substantial change in the caries experience of these children in this relatively short period of time. Accordingly, individual histories of exposure to the fluoridated water were not obtained and the influence of fluoridation as a subject of inquiry was eliminated from the 1955 survey. Consequently the results of this survey cannot properly be interpreted with reference to the fluoridation program.

PROCEDURE

The primary sampling units in the 1955 survey were elementary public schools with an October, 1954 enrollment of 300 or more children of the same race. In the case of white children, the schools were stratified in 5 socioeconomic categories, determined by the 1950 median rental of housing units in the census tract wherein the school was located. These categories were defined by the following ranges of monthly rental: 20 to 32, 33 to 40, 41 to 47, 48 to 56, and 57 to 97 dollars. The survey was made in 3 schools randomly selected from each range, that is, in 15 schools with enrollments of white children. From each school, 20 boys and 20 girls in each of the age groups of 6, 8, and 10 years last birthday were chosen at random from a master list of all the pupils in these ages. In this manner, 1,800 boys and girls of the white race were made subjects of study.

Received for publication July 9, 1956.

In the case of nonwhite children, socioeconomic stratification was not practicable. Therefore a random sample of 6 schools with Negro enrollments was selected, from which subsamples of 20 boys and 20 girls at each age were chosen in the same way as among the white children, giving a total of 720 children of the Negro race used as subjects in the study.

Inspections.—All the children were inspected in the school by the same dentist, using mirror, explorer, and good illumination. The dentist obtained the number of permanent teeth erupted, decayed, filled, decayed and filled, indicated for extraction, and extracted. Eliminating the number of erupted teeth, the sum of these mutually exclusive elements comprised the caries experience (DMF) score for the child. Immediately after the teeth were inspected, each child received an oral hygiene score which expressed the relative cleanliness of the teeth and mouth. This score was derived from an estimation of plaque material on the 6 maxillary anterior teeth, using simple criteria for the assignment of a number for each.[2] Individual teeth were scored from 1 to 4, the highest representing a relatively high state of cleanliness and freedom from surface deposits. All children were scored by the same dental hygienist without knowledge of the dentist's findings.

RESULTS

The findings of the 1955 survey are presented and compared with 1952 data in Table I. The per cent of 6-, 8-, and 10-year-old white children who had experienced a caries attack in the permanent teeth in 1955 was virtually the same as in 1952, but among Negro children it appeared to have increased. Similarly the number of decayed, missing, and filled (DMF) permanent teeth in white children in 1955 was about the same as in 1952, but in Negro children it also was increased. The earlier survey indicated an average of 2.8 DMF teeth per child in combined 6-, 8-, and 10-year-old white children and 1.8 in similarly aged Negro children. In 1955, the parallel values were 2.9 in the white and 3.1 in the nonwhite group.

The increased prevalence of tooth decay among Negro children may be more apparent than real, particularly in view of the failure of white children to exhibit a similar increase. There is reason to presume that the design of the 1952 survey contributed to the discrepancy. In 1952 two teams of dentists performed the inspections. Six white dentists inspected the white children and 4 Negro dentists the Negro children. In 1955, one dentist observed both groups. Therefore it is entirely possible that the 1952 findings were influenced by a bias stemming from subjective differences in the appraisal of tooth decay by different dentists, which may occur whenever the probability of being inspected by a given dentist is not the same for all the children in the study. On the other hand, the 1955 results are necessarily free of this type of bias. Consequently differences in decay experience between white and Negro children recorded in 1952 may have been unreal and the races as comparable in this respect in 1952 as the 1955 survey would indicate.

EPIDEMIOLOGY

Age, Sex, and Race.—As shown in Table I, whether expressed in DMF teeth per child or per 100 teeth erupted, no appreciable differences in caries experience existed between boys and girls or white and Negro children studied in 1955. The apparent reversal of the increase in decay with age observed among 10-year-old children under "Mean Number of DMF Teeth per 100 Teeth Erupted" does not imply a reversal of caries experience but rather an increase in the denominator of the DMF index occasioned by the recent eruption of canine and premolar teeth.

TABLE I
CARIES EXPERIENCE IN PERMANENT TEETH
WHITE AND NEGRO CHILDREN 6, 8, AND 10 YEARS OF AGE
BALTIMORE, 1955 AND 1952

	AGE LAST BIRTHDAY	NUMBER OF CHILDREN		PER CENT OF CHILDREN HAVING ONE OR MORE DMF PERMANENT TEETH		MEAN NUMBER OF ERUPTED PERMANENT TEETH PER CHILD		MEAN NUMBER OF DMF TEETH PER 100 TEETH ERUPTED		MEAN NUMBER OF DMF TEETH PER CHILD	
		1955	1952	1955	1952	1955	1952	1955	1952	1955	1952
White boys	6	300	257	43	38	5.1	4.0*	20.4	17.9*	1.1	0.9
	8	300	361	88	82	11.0	10.7*	27.4	26.5*	3.0	2.7
	10	300	259	98	95	16.9	14.8*	24.5	26.7*	4.1	4.1
White girls	6	300	240	50	41	5.7	5.0*	21.1	18.2*	1.2	1.0
	8	300	307	91	90	12.2	11.8*	26.6	23.2*	3.2	3.1
	10	300	309	99	93	18.7	16.3*	24.6	25.2*	4.6	4.4
Negro boys	6	120	87	50	14	6.3	5.8	19.1	4.5	1.2	0.3
	8	120	120	87	77	11.6	11.1	26.1	16.9	3.0	2.2
	10	120	181	99	82	18.5	19.0	25.2	13.4	4.7	2.5
Negro girls	6	120	126	54	15	6.6	6.9	21.3	5.4	1.4	0.3
	8	120	135	96	69	12.7	12.5	27.7	14.3	3.5	1.8
	10	120	203	96	85	20.6	21.3	21.8	12.1	4.6	2.6

DMF teeth are teeth with a caries experience—decayed, missing, and/or filled.
*Mean derived from group about one half the size indicated under "Number of Children," 1952. Erupted teeth were counted in only about half the white children in the 1952 survey. No attempt was made to select children for this purpose.

Socioeconomic Status.—In 1955, the caries experience of 360 combined 6-, 8-, and 10-year-old white children in each of 5 socioeconomic classes, expressed as the mean number of DMF permanent teeth per 100 teeth erupted, and given in the order of improving socioeconomic grade, was 25.0, 23.6, 25.3, 25.3, and 21.3. No definite relationship was apparent between the prevalence of tooth decay and socioeconomic status.

Oral Hygiene.—Oral cleanliness scores were obtained in 1,783 of the 1,800 white and 715 of the 720 Negro children studied in 1955. The distribution of the findings of DMF teeth in these children, classified by their oral hygiene scores, is shown in Table II. An inverse relationship was observed between the prevalence of tooth decay and the index of oral cleanliness in every age and race group where the number of children was sufficient to obtain a dependable count

TABLE II
MEAN NUMBER OF DMF PERMANENT TEETH PER CHILD
ACCORDING TO ORAL HYGIENE INDEX
WHITE AND NEGRO CHILDREN, 6, 8, AND 10 YEARS OF AGE
BALTIMORE, 1955

	AGE OR RACE		ORAL HYGIENE SCORES*			
			1.0-1.9	2.0-2.9	3.0-3.9	4.0
Combined races	6	Children	4	46	364	410
		Mean DMF	0.4	1.3	1.2	1.0
	8	Children	8	95	430	302
		Mean DMF	3.5	3.1	3.0	2.8
	10	Children	18	106	444	271
		Mean DMF	5.4	4.7	4.2	4.0
Combined ages	White	Children	27	173	867	716
		Mean DMF	4.3	3.3	2.8	2.3
	Negro	Children	3	74	371	267
		Mean DMF	4.5	3.9	3.0	2.5
	All	Children	30	247	1,238	983
		Mean DMF	4.4	3.5	2.9	2.4

*A child's oral hygiene score is the sum of the scores of the individual maxillary anterior teeth divided by the number of those teeth present.

of DMF teeth. The remarkable consistency of this finding warrants further investigation of the apparent influence of tooth cleanliness in children of these and other ages. There was no evidence that oral hygiene scores were associated with a socioeconomic gradient.

DENTAL CARE

Collection of the data from different socioeconomic levels provided an opportunity to assess the existing amount of dental care and the need for dental care relative to this axis of classification. As an index of care, the average number of filled, including decayed and filled, permanent teeth per 100 permanent teeth

TABLE III
MEAN NUMBER OF FILLED PERMANENT TEETH* PER 100 PERMANENT TEETH ERUPTED
ACCORDING TO AGE, SEX, RACE, AND SOCIOECONOMIC STATUS
BALTIMORE, 1955

AGE	SEX	WHITE CHILDREN SOCIOECONOMIC LEVELS LOW 1	2	3	4	HIGH 5	COMBINED WHITE CHILDREN	COMBINED NEGRO CHILDREN
6	Boys	0.7	3.2	0.0	0.0	1.0	1.4	1.1
	Girls	1.9	2.6	0.8	2.1	1.3		
8	Boys	2.0	3.2	1.0	4.8	9.4	4.3	0.8
	Girls	1.2	6.3	2.0	6.0	7.0		
10	Boys	1.5	3.8	3.6	4.7	9.1	5.6	1.0
	Girls	3.0	5.5	5.0	6.5	13.1		
All		1.9	4.4	2.8	4.9	8.7	4.5	0.9

*Including teeth decayed and filled; that is, teeth having both a filling and an unfilled carious lesion in the same tooth.

erupted in the children surveyed in 1955 is shown in Table III. Significance differences ($p \leq 0.05$) occurred between white children of different socioeconomic levels, and between the white and the Negro children. In the ages 6 to

10, a definite increase in filled teeth with increasing age was observed among white children, and in general, girls received more dental care than boys. For combined ages the average number of filled teeth per hundred teeth in boys was 2.8 and in girls 3.9, with a standard error of 0.3. Excepting socioeconomic Group 2, the frequency of filled teeth was greater in children from higher socioeconomic levels. Probably Group 2 children failed to conform to the trend as a consequence of the activity of a vigorous dental treatment program operated by the Baltimore City Health Department in 2 of the 3 schools of this group.* In Negro children there was about 1 filled permanent tooth per 100 teeth erupted at any age, although the decay experience was not materially different from that of the white children.

Needs.—The unmet need for dental care in the same children, expressed by the per cent of permanent teeth with a caries experience (DMF teeth) that required filling or extraction, is indicated in Table IV. Except in socioeconomic

TABLE IV
PER CENT OF DMF PERMANENT TEETH THAT REQUIRED FILLING OR REMOVAL ACCORDING TO AGE, SEX, RACE, AND SOCIOECONOMIC STATUS
BALTIMORE, 1955

AGE	SEX	WHITE CHILDREN SOCIOECONOMIC LEVELS LOW 1	2	3	4	HIGH 5	COMBINED WHITE CHILDREN	COMBINED NEGRO CHILDREN
6	Boys	93	83	100	93	98	92	93
	Girls	92	90	93	94	93		
8	Boys	94	86	93	81	62	83	96
	Girls	93	76	91	72	72		
10	Boys	90	79	84	80	60	75	93
	Girls	84	75	73	69	41		
All		91	81	89	81	71	83	94

Group 2, which apparently reflected the influence of the Health Department dental program,* there was little evidence of dental intervention in any children of the 6-year age group. At age 8 there appeared a tendency for unmet needs to be reduced among white children in the higher socioeconomic grades, which at age 10 was well established, with girls showing greater reductions than boys. Unlike those of white children, the dental care requirements of Negro children were high regardless of age or sex.

A substantial necessity for dental treatment was evident among all the children, including those in good socioeconomic circumstances. The lowest index of need, 41 per cent, occurred in 10-year-old girls in the highest socioeconomic grade studied. Whether the fault lies in the failure of dental health education, parental shortcoming, or in dentists loathe to accept child patients, is conjectural. Certainly there is reason for encompassing children from every level of the population in school dental programs.

*In the 3 schools of socioeconomic Group 2, the 120 children from the 1 not in the program had 2.0 filled permanent teeth per 100 permanent teeth erupted while the 240 children from the 2 in the program had 4.9 filled permanent teeth per 100 permanent teeth erupted.

SUMMARY

A 1955 survey of 2,520 Baltimore public school children 6, 8, and 10 years old confirmed the findings of caries experience observed among white children in 1952. A difference in the prevalence of the disease between white and Negro children recorded in 1952 was not duplicated when one dentist examined the children of both races. Children living in relatively good socioeconomic circumstances experienced no less dental caries than those in meaner circumstances. An inverse relationship was found between caries experience and an index of oral cleanliness. Dental care in 8- and 10-year-old white children was related to their socioeconomic status but Negro children received very little care at any age. Unmet needs for dental treatment were substantial at every population level.

The authors gratefully acknowledge the help of Baltimore's public school officials, principals, teachers, and children, without whom this study could not have been made. Thanks are due also to Dr. L. Paul Rivas, the dentist who inspected all the teeth, to Mrs. Anne Fink Jacobs, the dental hygienist who provided the oral hygiene scores, and to Mr. Raleigh Cline, who served as statistical aide.

REFERENCES

1. McCauley, H. B., and Frazier, T. M.: The 1952 Dental Survey in Baltimore, Maryland, Quarterly Statistical Report of the Baltimore City Health Department **6**: 3-18, 1954.
2. King, R. M.: A Clinical Study of the Effect of Tetradecylamine on Plaque Formation, *J. D. Res.* **30**: 399-402, 1951.

THE METABOLISM OF NATURAL AND ARTIFICIAL FLUORIDATED WATERS

MARTIN J. WAGNER AND JOSEPH C. MUHLER

Indiana University, Department of Chemistry, Bloomington, Ind.

COMMUNAL water fluoridation is now considered to be an acceptable public health procedure for partially controlling dental caries in children. There are recognized deficiencies in communal fluoridation, such as its unavailability to rural children, its reduced effectiveness when initiated in children over 6 years of age and the fact that the majority of the fluoridated water in any community is not used for purposes which would be therapeutic. Notwithstanding these facts, water fluoridation is the most efficient and least costly anticariogenic procedure, and the one which has been repeatedly demonstrated to have the highest level of effectiveness of any method yet developed.

There have been serious objections raised by some workers to the artificial* fluoridation of communal water supplies which contain less than 1 μg of fluorine per milliliter (1 ppm) as a natural constituent. These objections are based, in part, upon the assumption that the fluorine furnished to the water supplies by "nature" is more effective in preventing tooth decay than the "artificial" addition of sodium fluoride. Moreover, it has been contended that there are pronounced differences in the metabolism of "natural" as opposed to "artificial" fluorides. It is claimed that less "available" fluorine is present in the water from areas where there is natural fluorine present than when sodium fluoride is artificially added to a natural fluoride-deficient water, with the result that there is less toxicity in naturally fluoridated waters. Even though recent medical studies have failed to show any significant differences in the health of people drinking artificially fluoridated water as compared to similar people using a fluorine-deficient water supply, the objections continue to serve as deterrents for many communities which are interested in fluoridating their water supplies. Therefore, this study was designed in an attempt to determine if the metabolism of fluorine in rats is significantly different if they receive identical amounts of fluorine furnished in water from a natural source, or from water obtained by adding sodium fluoride to mineral-free water. It was of most immediate interest to make this comparison between waters containing fluoride at the level of 1.0 μg per milliliter, the concentration most used in fluoridating communal waters. Since this fluoride

This work was supported in part by a grant from the Indiana State Board of Health, Indianapolis, Ind.

Received for publication March 29, 1956; revised April 2, 1957.

*The use of the word "artificial" may not be appropriate in this instance. Perhaps a better word would be "fortify." Thus, one would fortify the fluorine-deficient water supplies on the grounds that one is replacing a natural constituent of the water. Similar reasoning applies to the "fortification" of cereal grains with certain vitamins which have been lost during the milling process.

concentration is quite low, it would be possible that the interference of calcium and magnesium salts known to be present in water supplies and which are known to interfere with fluoride metabolism when present at higher concentrations[1] would not be evident. It was therefore considered advisable to include a second series of data obtained in a comparison of the metabolism of fluoride obtained from natural or artificially fluoridated waters containing a level of fluoride approximately 8 times as high as in the first series.

EXPERIMENTAL

In these studies 8 litters of weanling Sprague-Dawley strain rats were used. At the beginning of the study sufficient animals were sacrificed for the determination of initial carcass fluoride. This is necessary so that the total amount of fluoride found in the whole animal at the termination of the experiment can be related to the total fluoride intake during the study. By subtracting the amount of fluoride found at the initiation of the study from that at the termination of the experiment, that amount retained during the study is found. Each litter was then divided by sex and body weight into 2 groups. One group was designated AF (artificially fluoridated water), the other NF (natural fluoride water). Each animal was housed individually in a raised screen cage in a temperature-controlled room. Low fluoride diet* (F = 0.3 ppm) was available ad libitum. No attempt was made to measure routinely the amount of diet consumed by the animals because of its low absolute fluoride content as well as the metabolic inertness of the dietary fluoride. It was felt also that comparisons between the groups in any litter would be unaffected by the amount of diet eaten since, on the average, both groups would consume approximately the same amount.

Four litters, constituting Series I, received fluoride drinking water containing fluoride at a concentration of approximately 1.1 μg per milliliter. Within each litter the natural fluoride (NF) group received drinking water obtained from Decatur, Indiana. This was ordinary community drinking water, quantities of which were secured over a period of 8 weeks in order to obtain representative water samples for the community. The fluoride concentration was 1.11 ± 0.02 μg per milliliter. The artificial fluoride (AF) animals received drinking water prepared from redistilled fluorine-free water and reagent grade sodium fluoride. The fluoride concentration was matched to that of the natural fluoride water and was determined periodically to be 1.09 ± 0.03 μg per milliliter. Both waters were stored in paraffined glass carboys in order to retard absorption of fluoride by the glass.

The animals comprising the 4 litters of Series II received water containing approximately 8.0 μg F per milliliter. The NF animals received untreated city water from the water supply of Bartlett, Texas. The fluoride concentration of this water was determined to be 8.0 ± 0.4 μg per milliliter. This water

*Corn meal, 64.0%; alfalfa meal, 4.8%; irradiated yeast, 0.2%; iodized sodium chloride, 1.0%; and powdered whole milk, 30.0%

was received and stored in a polyethylene carboy. The AF animals received water prepared as for Series I, but matched to the higher fluoride level of the Texas water.

Within each litter of both series, all animals received identical measured quantities of water each day. The quantity was increased as rapidly as the drinking capacity of the animals would permit. Water spilled from the drinking tube was funneled into a covered beaker through a modified thistle tube acting as a catch basin below the drinking tube. The spilled and residual water was measured and recorded for every animal each day. Thus, through paired drinking technics it was possible to assure the ingestion of identical quantities of the natural and artificially fluoridated water. If any animal finished his fluoride-water portion before the end of the 24-hour day, he received redistilled fluoride-free water for the remainder of that period.

Metabolism data were obtained from all but one litter. For this purpose animals from each group were housed in round, stainless steel cages having a 2-mesh floor. The separate analysis of daily urine and feces was compared to the aqueous fluoride intake for the same length of time. These methods have been described previously.[2]

The total balance study conducted in one litter of Series II applied the procedures described above to the entire experimental period. In addition, the diet consumption of each animal was measured daily.

All litters were killed at the end of 40 days, with the exception of the Series II total balance litter, which was killed after 17 days. The skinned and ashed carcass was then analyzed for fluoride content by methods described previously.[2]

RESULTS AND DISCUSSION

The results of these studies are presented in Table I. The table shows the total amounts of fluoride provided by the drinking water, the amount and concentration of the carcass fluoride and the percentage retention of fluoride based on the aqueous fluoride ingested. The data suggest a tendency toward greater retention of fluoride by the skeleton when obtained from a pure sodium fluoride solution. This trend is much more pronounced in Series II, although in neither series are the differences significant.

The high percentage of ingested fluorine retained by the carcass may be explained by consideration of the age of the animals used in these studies, since they were weanling rats and had no previous exposure to fluorine. It is to be expected that under these conditions the retention of fluorine should have been high as a result of the rapid growth of the skeleton.

The differences in retention, if they are real, may depend on many factors. Important among these may be differences in absorption of the fluoride from the 2 sources by the gastrointestinal mucosa. Since the presence of inorganic elements in the diet or water is known to decrease fluoride absorption,[1] these data would suggest that the inorganic elements present in the natural water and not present in the artificial fluoride source may interfere with fluoride

TABLE I
RESULTS OF CARCASS ANALYSIS OF ANIMALS RECEIVING AQUEOUS FLUORIDE FROM A NATURAL (NF) AND AN ARTIFICIAL (AF) SOURCE

LITTER	GROUP	NO. OF RATS	TOTAL FLUORIDE INGESTED (μG)	CARCASS FLUORIDE μG	CARCASS FLUORIDE PPM	FLUORIDE RETENTION (%)
			Series I (1.1 ppm F water)			
1	AF	3	658 ± 1.5	463 ± 75	95 ± 6	70
	NF	3	655 ± 6.5	457 ± 11	93 ± 2	69
2	AF	4	703 ± 25.9	596 ± 17	123 ± 3	85
	NF	4	712 ± 0.3	586 ± 18	133 ± 4	82
3	AF	5	664 ± 7.9	500 ± 6	116 ± 2	75
	NF	5	656 ± 11.4	493 ± 25	104 ± 3	75
4	AF	4	698 ± 1.9	634 ± 27	134 ± 2	91
	NF	4	707 ± 3.3	607 ± 7	142 ± 7	86
			Series II (8.0 ppm F water)			
1	AF	3	5530 ± 10	3300 ± 168	637 ± 20	60
	NF	4	5750 ± 10	3555 ± 222	645 ± 27	58
2	AF	3	4200 ± 17	3814 ± 68	638 ± 17	91
	NF	3	4350 ± 0	3684 ± 44	665 ± 45	85
3	AF	3	1500 ± 6	1039 ± 30	282 ± 9	71
	NF	3	1563 ± 12	1002 ± 29	282 ± 16	64
4	AF	3	3923 ± 22	2003 ± 101	392 ± 41	59
	NF	3	3067 ± 3	1856 ± 70	424 ± 27	61

solubility and thus its availability. This would be a reasonable conclusion, since the artificial fluoride water was made from redistilled, mineral-free water. This increased retention of fluoride occurring from the artificial water should not be interpreted to mean that this would occur in a community fortifying its water supply to the level of 1 μg per milliliter with fluoride, since such water would in all probability be similar in its major inorganic composition to that of the natural water used in these studies.

The results of metabolism studies are shown in Table II. Data given have been obtained from 2 animals per group except in Series II, litters 3 and 4, in which 3 animals per group were studied. In Series II the per cent excretion by the natural fluoride groups is greater than that of the corresponding artificial fluoride groups in all but one litter. This correlates well with the pattern of skeletal retention for these animals reported in Table I, in which the natural fluoride groups had proportionately less skeletal fluoride at the termination of the experiment than the animals receiving the artificial fluoridated water.

It is interesting to note the pattern of fluorine excretion in litter 4 of Series II. In this instance, metabolism data were obtained at 3 different age periods during the experiment. During the first period, nearly halfway through the study, the animals were excreting averages of 22 and 26 per cent of the ingested fluoride for the artificial and natural fluoride groups, respectively. The last metabolism samples analyzed were obtained just prior to the termination of the study, and show an approximate 100 per cent increase in the quantities of fluoride being excreted, in relation to the amount of fluoride ingested. Thus it would appear that even in young animals with no previous

fluorine experience, a resistance to further storage soon appears as exposure continues. Increasing fecal fluorine throughout the period is simply a reflection of greater diet intake as the animal increases in size. However, the urinary fluoride increased beyond a simple proportionality with the aqueous fluoride intake. Here it appears that skeletal uptake of fluoride is another manifestation of the law of mass action, by which further uptake is resisted more strongly as stored fluoride increases. This phenomenon is seen to a lesser degree in the metabolism studies of litter 1, Series I.

TABLE II
METABOLISM STUDIES OF RATS RECEIVING AQUEOUS FLUORIDE FROM A NATURAL (NF) AND ARTIFICIAL (AF) SOURCE

LITTER	GROUP	DAYS STUDIED DURING PERIOD	F INGESTED (MEAN/DAY) (μG)	F EXCRETION (MEAN/DAY) URINE (μG)	FECES (μG)	TOTAL (μG)	% EXCRETION
Series I (1.1 ppm F water)							
1	AF	13-15	15.5	3.5	1.8	5.3	34.2
	NF		15.2	3.8	1.1	4.9	32.2
2	AF	36-38	25.3	11.5	2.7	14.2	56.1
	NF		23.5	10.7	2.5	13.2	56.0
3	AF	36-38	29.6	13.1	2.6	15.7	53.1
	NF		26.2	11.9	2.0	13.9	52.9
4	AF	37-39	25.0	12.0	2.4	14.4	57.5
	NF		22.0	11.3	1.3	12.6	57.3
Series II (8.0 ppm F water)							
1	AF	36-40	148	68.2	5.1	73.3	49.5
	NF		158	67.9	4.0	71.9	45.5
2	AF	30-40	147	55.2	3.2	58.4	39.7
	NF		149	59.6	17.1	76.7	51.5
3	AF	1-17	84	15.2	4.7	19.9	23.7
	NF		88	16.9	4.6	21.5	24.4
4	AF	17-19	89	17.1	2.4	19.5	21.9
	NF		74	17.3	2.2	19.5	26.4
4	AF	30-33	73	23.8	3.3	27.1	37.1
	NF		74	27.8	4.5	32.3	43.6
4	AF	37-40	64	21.2	7.5	28.7	44.8
	NF		68	22.8	8.5	31.3	46.0

In litter 3 of Series II a total balance study was conducted. This was designed to answer the question concerning the magnitude of the contribution of dietary fluoride, as well as to enable a balancing of excretion against retention for the entire experimental period. It had been felt in previous work that perhaps metabolism studies of isolated times lasting 3 to 5 days out of the entire period might not satisfactorily reflect the metabolism trends of the entire period. The conclusions obtained from this total balance study did not confirm these suspicions. Table III shows the amounts of fluoride contributed by drinking water and diet for the 17-day period, the total fluoride excreted, and the final carcass values. The urine and feces were analyzed separately each day, and diet and water consumption was measured daily. Carcass fluoride values in this as in all other litters are the means of duplicate analyses. The average difference between total fluoride given and total fluoride found in carcass and excretion amounts to 9 per cent of the average total fluoride

intake, a value reflecting good precision considering the number of analyses performed, the reliability of the analytical method, and the amount of fluoride present in the metabolism samples.

TABLE III

TOTAL BALANCE STUDY OF RATS RECEIVING AQUEOUS FLUORIDE FROM A NATURAL (NF) AND ARTIFICIAL (AF) SOURCE (LITTER 3, SERIES II)

GROUP	ANIMAL	F INITIAL (μG)	F INGESTED			TOTAL F EXCRETED (μG)	F IN CARCASS (μG)	△ F (GIVEN-RECOVERED) (μG)
			WATER (μG)	FOOD (μG)	TOTAL (μG)			
AF	1	10	1430	45.0	1475	337.4	1099	39
	2	10	1424	48.5	1473	376.6	1010	86
	3	10	1422	45.7	1468	296.9	1009	162
NF	1	10	1509	47.7	1557	424.9	945	187
	2	10	1500	48.3	1548	338.0	1026	184
	3	10	1478	43.8	1522	333.2	1036	153

Table IV tabulates for each animal in litter 3 the per cent excretion, per cent retention, and the sum of the 2, all expressed on the basis of total fluoride ingestion. These data again suggest that only about 20 to 25 per cent of the ingested fluoride is excreted by the young rat. It is frequently stated that upwards to 90 per cent of the ingested fluoride is excreted by human beings. McClure[3] stated, for example, "The results of fluorine balance studies are interpreted as being strongly indicative that quantities of fluorine up to 3.0-4.0 mg. ingested daily are perhaps more than 90 per cent eliminated by the average adult." Since these and other data are in contrast to this statement, additional work is needed in order to clarify the problem. Obvious differences between human and rat studies are immediately apparent, and the effects of age of animals, previous experiments with fluorine, the type of fluoride compound, and the effects of the diet need to be carefully evaluated before any comparison between these data and the conclusions of McClure is possible.

TABLE IV

EXCRETION AND RETENTION OF FLUORINE IN ANIMALS OF LITTER 3, SERIES II, EXPRESSED AS PER CENT OF TOTAL FLUORIDE INGESTED

GROUP	ANIMAL	% EXCRETION	% RETENTION	% EXCRETION PLUS % RETENTION
AF	1	22.9	74.5	97.4
	2	25.6	68.5	94.1
	3	20.2	68.8	89.0
NF	1	27.3	60.7	88.0
	2	20.8	66.3	87.1
	3	21.8	68.0	90.8

CONCLUSIONS

The ingestion, by weanling rats, of an artificially fluoridated water containing fluoride at 2 different levels results in no significant difference in skeletal retention when compared to rats given a natural fluoride water of

corresponding fluoride levels. The numerical differences in retention, however, are much more pronounced in the series of animals receiving the higher fluoride level water. The differences may be ascribed to interference with absorption of fluoride by the inorganic constituents of the natural water.

The proportion of ingested fluoride that is excreted increases rapidly as the animal is exposed to the element. This increase does not appear directly proportional to the concentration of fluoride ingested.

In a total balance study, recovery of fluoride can be quantitative enough to give complementary results in calculation of ingestion, excretion, and skeletal retention.

REFERENCES

1. Weddle, D. A., and Muhler, J. C.: The Effects of Inorganic Salts on Fluorine Storage in the Rat, *J. Nutrition* **54**: 437, 1954.
2. *Idem:* Metabolism of Small Concentrations of Fluorine in the Albino Rat, *J. D. Res.* **34**: 900, 1955.
3. McClure, F. J.: Nondental Physiological Effects of Trace Quantities of Fluorine in Dental Caries and Fluorine, editor, F. R. Moulton, Washington, D. C., 1946, Am. Assoc. Advancement Sc., p. 89.

THE EFFECTS OF SALIVARIADENECTOMY ON THE REPRODUCTIVE ORGANS OF THE FEMALE RAT

DAVID BIXLER, PH.D., RICHARD C. WEBSTER, PH.D., AND JOSEPH C. MUHLER, D.D.S., PH.D.

Indiana University, Bloomington, Ind.

PREVIOUS reports have suggested that the removal of the major salivary glands (salivariadenectomy) may effect the weight and histologic structure of the adrenal glands and reproductive organs.[1-4] The purpose of this paper was to define the effects of removal of the major salivary glands upon the pituitary-gonad system and to determine to what extent the pituitary gonadotropic hormones might be involved.

EXPERIMENTAL

The effects of salivariadenectomy were investigated in 3 series of experiments. Series I was concerned with effects of salivariadenectomy upon the weights of the male and female reproductive organs. In Series II, the histology of the female reproductive organs was studied at varying intervals of time following salivariadenectomy. Series III was concerned with the assay of pituitary gonadotropic hormones in salivariadenectomized animals.

Series I was composed of 18 male and 16 female rats equally divided into salivariadenectomized and control groups. The animals were fed a stock diet* for the experimental period of 40 days. At this time they were sacrificed and the ovary, uterus, testes, and seminal vesicles were removed and weighed.

A total of 100 female weanling rats (Sprague-Dawley strain), which comprised Series II, were equally divided into 4 experimental groups. Group I was salivariadenectomized.[5] Groups II, III, and IV were unoperated and differed from each other only in their daily food consumption. Each animal in Group II was restricted to the daily food intake of its paired mate in Group I, while the animals in Group III were placed on a chronic caloric restriction of two thirds the amount consumed by their respective pair-mate in Group I. The animals in Group III, while underfed, were still able to gain weight although at a markedly reduced rate. Group IV animals were fed ad libitum and served as controls. All animals were given distilled water ad libitum. They were housed in raised screen cages in an air-conditioned room.

Two animals from each of the 4 groups were sacrificed by a blow on the head at 6 hours, 1, 2, 4, 8, 16, 25, 32, and 60 days following the time of operation of Group I. At this time, the uteri and ovaries were removed, fixed in formalin

Received for publication June 8, 1956.

*Diet composed of 53% surcrose, 31% powdered whole milk, 10% cottonseed oil, 4% salt mixture, 1% vitamin mixture, 1% whole wheat and 15 drops of Oleum Percomorphum per kilogram of diet.

and sectioned serially at 8 microns. Attempts to measure the estrus cycle of these animals by vaginal smears were unsuccessful, except in the 60-day group. Apparently the younger animals did not have an established estrus cycle.

In Series III, 400 weanling female rats were equally divided into 2 experimental groups according to initial body weight. One group was salivariadenectomized and the other was unoperated and served as a control. All animals were fed the diet described previously for Series I. Twenty-five days after the operation, all animals were sacrificed by decapitation, the pituitary glands and ovaries weighed, and the pituitaries placed in a large excess of acetone until assayed. For assay purposes, 40 female albino rats which had been hypophysectomized at 40 days of age were used.* These animals were fed ad libitum a special diet of carrots, oranges, dog meat, bread, and milk.

The pituitaries taken from both groups were prepared for assay by decanting the acetone and after drying they were weighed en masse. Water was added to make the desired concentration of tissue. Sodium chloride was added in order to make the solution physiologic, and the glands homogenized. The homogenates of the 2 groups were injected intraperitoneally into the hypophysectomized animals once each day for 3 consecutive days. In all, 6 groups were injected, respectively, with the equivalent by weight of 1, 2, 3, 4, 5, and 6 fresh pitutiaries from the salivariadenectomized animals. Six additional groups received the same graded amounts of pituitary tissue from control animals. Twenty-four hours after the last injection the assay animals were sacrificed and the ovaries and uteri removed for histologic examination.

The sella turcica was carefully examined for incomplete removal of the hypophysis and animals with any remnant of tissue were discarded. Only 4 animals were discarded for this reason.

RESULTS AND DISCUSSION

Series I.—This experiment, in which the ovarian, uterine, testicular, and seminal vesicle weights of salivariadenectomized rats were determined, revealed a statistically significant reduction in weight of each of these organs 50 days after the operation. These data are shown in Table I. These results were the stimulus for a histologic study of the female reproductive organs at varying time intervals and this experiment was designated Series II.

TABLE I

EFFECT OF SALIVARIADENECTOMY UPON REPRODUCTIVE ORGAN WEIGHTS (SERIES I)

	RATS	TESTIS OR OVARY (MG. %)	P	SEMINAL VESICLE OR UTERUS (MG. %)	P
Male					
Control	9	1388	—	134.8	—
Salivariadenectomized	9	1207	.03	98.3	.01
Female					
Control	8	55.8	—	167.1	—
Salivariadenectomized	8	43.6	.008	109.5	.02

*Obtained from Hormone Assay Laboratories, Chicago, Ill.

Series II.—The histologic findings in the ovaries of the 4 experimental groups are summarized in Table II. Examination of the ovaries of the unoperated control and pair-fed groups revealed no significant differences throughout the entire experimental period of 60 days. For the first 25 days, both groups had ovaries which contained numerous follicles in all stages of development, some of which were mature but undergoing atresia. At the twenty-fifth day (55 days of age), corpora lutea first appeared and approximately 6 to 8 were present at each subsequent period up to 90 days of age. Mitoses were observed in the granulosa cells of the follicles during this latter period and the interstitial cells had nuclei with evenly dispersed chromatin and a normal-appearing cytoplasm (Fig. 1, *A* and *B*).

TABLE II

COMPARISON OF EFFECTS OF SALIVARIADENECTOMY AND CHRONIC INANITION ON OVARY OF RAT (SERIES II)

GROUP	EXPERIMENTAL DAY	INTERSTITIAL LUTEINIZATION	AVERAGE NUMBER OF CORPORA LUTEA	FOLLICULOGENESIS
Salivariadenectomized	25*	–†	0	+++†
	32	+	2	++
	60	++	3	+++
Pair-fed	25	++	2	+++
	32	+++	5	+++
	60	+++	8	+++
Restricted diet	25	–	0	–
	32	–	0	–
	60	–	0	+
Control	25	++	3	+++
	32	+++	6	+++
	60	+++	8	+++

*Corresponds to an animal age of 55 days.

†The following system of evaluation was utilized: – absent, no reaction; + slight reaction; ++ moderate reaction; and +++ normal, full reaction.

Up to the thirty-second experimental day, the ovaries of the salivariadenectomized animals also had follicles in all stages of development, although in comparison to those of the control animals, there appeared to be a greater number of small follicles (Fig. 1, *C*). Mature follicles were observed but they were all undergoing atresia. Corpora lutea did not appear in this group until the thirty-second day (62 days of age), and the number present was approximately one third that found in the control and pair-fed animals. Even at 90 days of age the corpora lutea were reduced both in size and number, and the interstitial tissue revealed the presence of atypical cells which had pycnotic nuclei. The ovary as a whole was not as well vascularized as in the control group and the interstitial tissue remained undeveloped.

The animals subjected to a moderate chronic inanition (Group III) had few follicles, none of which were mature. This lack of folliculogenesis prevailed throughout the entire experimental period. No corpora lutea were observed

and the organ as a whole had a markedly undeveloped appearance. The interstitial cells were hormonally unstimulated throughout the experiment and by the thirty-second experimental day the nuclear chromatin material had assumed a "wheel cell" appearance (Fig. 1, *D*).

The uteri of unoperated control and pair-fed animals progressed from a hormonally unstimulated organ with a low cuboidal epithelium and undeveloped

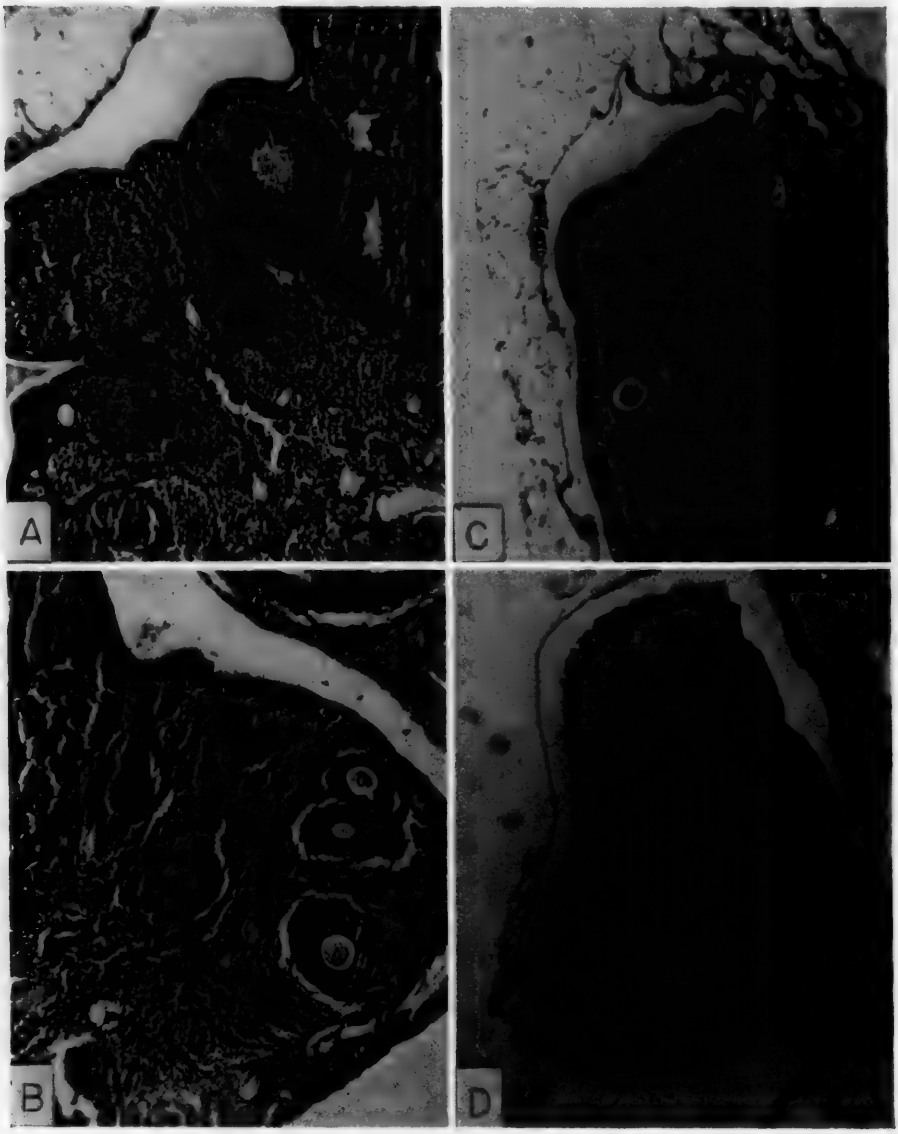


Fig. 1.—Microscopic appearance of ovaries from the 4 experimental groups at 90 days of age. (×90; reduced ⅓.) *A*, Control. *B*, Pair-fed. *C*, Salivariadenectomized. Note undeveloped interstitial tissue. *D*, Restricted diet. Entire ovary consisted of 2 follicles.

muscle layer at the fourth day, and a moderately stimulated organ at the twenty-fifth day, to one which was well developed and well vascularized by the sixtieth day (Fig. 2, *A* and *B*). The uterine epithelium at this time was a typical columnar type and the myometrium was composed of well-developed muscle cells. By contrast, the uteri of the salivariadenectomized animals were markedly retarded in development and even at the sixtieth experimental day the endometrium had a cuboidal epithelium and the myometrium was thin and characterized by small and undeveloped muscle cells (Fig. 2, *C*). The

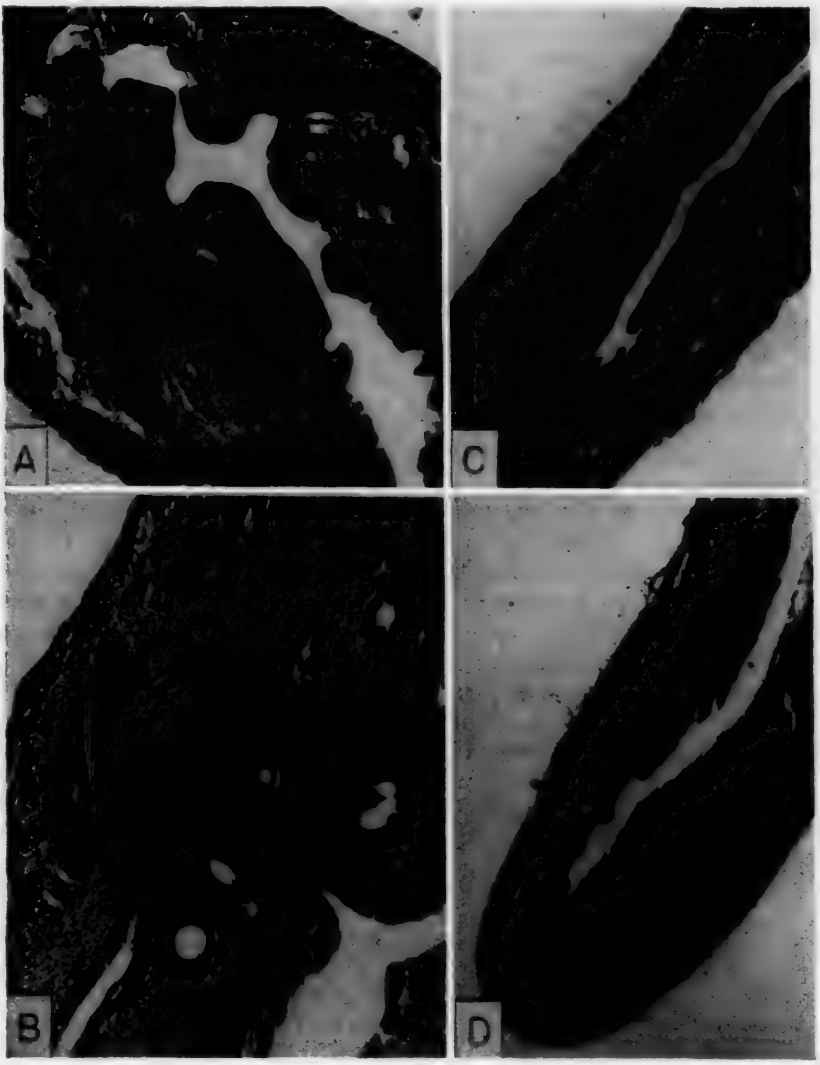


Fig. 2.—Microscopic appearance of uteri taken from the 4 experimental groups at 90 days of age. (×90; reduced ⅓.) *A*, Control. *B*, Pair-fed. *C*, Salivariadenectomized. Note small uterus size. *D*, Restricted diet. Note small uterus size.

uteri of the animals on a restricted diet remained undeveloped throughout the experiment and resembled those of castrated animals (Fig. 2, *D*).

Series III.—The organ weights obtained from the Series III animals appear in Table III. The decreased pituitary, ovary, and body weights of the salivariadenectomized rats (Table II) indicate that there is a general systemic growth failure in these animals. In addition, the ovarian weight differences shown here confirm the histologic findings of Series I, i.e., retarded development in the operated rats. In previous studies it was found that a sham operation had no effect on reproductive organ weights[3] or ovarian histology.[4] Similarly, the results of Series II and an earlier report[2] indicated that nutritional intake differences were probably not responsible for these reproductive organ changes. However, Ginn and Volker[6] reported an apparent vitamin B-complex deficiency in salivariadenectomized rats.

TABLE III

ORGAN AND BODY WEIGHTS OF SALIVARIADENECTOMIZED RATS 25 DAYS AFTER OPERATION (SERIES III)

GROUP	FINAL BODY WEIGHT (GM.)	PERCENTAGE OF ANIMALS WITH VAGINA OPEN	ORGAN WEIGHTS	
			PITUITARY (MG. %)	OVARY (MG. %)
Control (n = 193)	109 ± 15*	73.7	4.98 ± 0.93*	22.88 ± 4.81*
Salivariadenectomized (n = 183)	97 ± 15	35.3	4.52 ± 0.67	14.86 ± 4.76

*Standard deviation.

Table IV summarizes the results of the pituitary assay. The determination of a minimum effective dose of follicle-stimulating hormone (FSH) was based upon the appearance of: (1) medium- and large-sized follicles with a well-defined layer of granulosa cells, (2) mature Graafian follicles, and (3) mitotic figures in the granulosa cells. The following were used as criteria for a minimum response to luteinizing hormone (LH): (1) appearance of corpora lutea, and (2) luteinization of the thecal cells of the follicle. Using these criteria, it was found that the pituitary of salivariadenectomized rats had approximately 3 times the FSH content of the pituitary of comparable unoperated animals. Although histologic examination of the ovaries of Series II did not reveal any marked evidences of FSH deficiency, the ovarian weights (Table II) indicated that the pituitary gland of these operated animals is not releasing its FSH.

Similarly, the pituitary of the salivariadenectomized rats seemed to have a greater LH content. This conclusion is based upon what appeared to be a moderate luteinization of the interstitial tissue in the two groups receiving 5 or 6 pituitaries daily. However, it should be noted that no corpora lutea ever appeared in the ovaries of any of the 6 groups receiving pituitaries of the salivariadenectomized animals. The ovaries of the animals injected with the control pituitaries remained unchanged, in so far as an LH response was concerned, in all 6 groups. Possibly the injection of more pituitary tissue into

TABLE IV

GONADOTROPIC HORMONE CONTENT OF PITUITARY OF SALIVARIADENECTOMIZED FEMALE RATS (SERIES III)

	NUMBER PITUITARIES INJECTED PER DAY	NUMBER ANIMALS PER GROUP	FSH ACTIVITY	LH ACTIVITY
Control	1	4	S*	–
	2	4	S	–
	3	3	S	–
	4	3	S	–
	5	2	S	–
	6	2	M (MED)	–
Salivariadenectomized	1	4	S	–
	2	4	M (MED)	–
	3	3	L	–
	4	3	L	–
	5	2	L	+
	6	2	L	+

*Based on following system: Small follicles—S, medium follicles—M, and large follicles—L. MED signifies minimum effective dose.

these animals would have induced corpora lutea formation. Nevertheless, the salivariadenectomized rat's pituitary did elicit some LH response in the ovaries of both the 5 and the 6 pituitary groups.

These results indicated that the release of gonadotropic hormones from the pituitary of salivariadenectomized animals is inhibited. This could account for the retarded development of the ovary and uterus in these animals.

SUMMARY

Removal of the major salivary glands (salivariadenectomy) in the rat is followed by a retarded development of the female reproductive organs. This retarded development was characterized histologically by the appearance of a reduced number of mature follicles and fewer, atypical corpora lutea in the salivariadenectomized animals. Animals which were pair-fed to the operated animals demonstrated no ovarian or uterine changes. These results indicated that the effects of salivariadenectomy upon the reproductive organs were not entirely dependent upon nutritional differences.

Assay of the gonadotropic hormone content of the pituitary of salivariadenectomized rats indicated that the operation resulted in an increased amount of both FSH and LH in the pituitary gland. This result could explain the retarded development of the reproductive organs.

REFERENCES

1. Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries. II. Effect of Desalivation on Dental Caries and Castration and Desalivation on Fluorine Storage in the Rat, *J. D. Res.* **33**: 346, 1954.
2. Bixler, D., Muhler, J. C., and Shafer, W. G.: Effect of Desalivation on Adrenals, Uterus, and Testes in the Rat, *J. D. Res.* **34**: 910, 1955.
3. *Idem:* The Effects of Castration, Sex Hormones, and Desalivation on Dental Caries in the Rat, *J. D. Res.* **34**: 889, 1955.
4. Bixler, D., Webster, R. C., and Muhler, J. C.: The Histochemistry of the Adrenal Cortex Following Removal of the Major Salivary Glands, *J. D. Res.* **35**: 547, 1956.
5. Bixler, D., Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries V. The Effects of Desalivation and Castration on Caries and Fluorine Storage in the Rat, *J. Nutrition* **52**: 345, 1954.
6. Ginn, J. T., and Volker, J. F.: Rusting in Desalivated Albino Rats, *Endocrinology* **31**: 282, 1941.

THE EFFECT OF TESTOSTERONE, THYROXINE, AND CORTISONE ON THE SALIVARY GLANDS OF THE HYPOPHYSECTOMIZED RAT

DAVID BIXLER, PH.D., RICHARD C. WEBSTER, PH.D., AND JOSEPH C. MUHLER, D.D.S., PH.D.

Indiana University, Bloomington, Ind.

THYROID HORMONE and the sex hormones, particularly the androgens, may be responsible for maintaining the histologic structure of the rat submandibular gland.[1-4] Baker[5] reported that 3 to 6 mg. of ACTH given daily by continuous injection resulted in a striking hypertrophy of the intralobular ducts of the rat submandibular gland. Since the importance of the pituitary gland in these adrenal effects had not been investigated, it seemed desirable to study the salivary glands of hypophysectomized rats which had received adrenocortical hormone injections.

MATERIALS AND METHODS

A total of 48 female albino rats which had been hypophysectomized by the parapharyngeal approach at 40 days of age* were divided into 4 experimental groups. One group received daily subcutaneous injections of 10 μg of sodium thyroxine in aqueous solution and 250 μg of testosterone propionate in sesame oil. Group 2 was given daily intramuscular injections of 2.5 mg. of cortisone acetate in saline suspension. Group 3 was injected daily with 10 μg thyroxine, 250 μg testosterone, and 2.5 mg. of cortisone. Group 4 received no hormone injections and served as a control. All of the animals were fed ad libitum and received a special diet consisting of carrots, oranges, bread, dog meat, and milk for 21 days, at the end of which time they were sacrificed by ether. The salivary glands were removed and fixed in 10 per cent neutral formalin for subsequent histologic study. The glands were embedded in paraffin, sectioned at 5 microns, and the sections stained with either Mallory's triple connective tissue stain, for studying the granules of the intralobular ducts and serous acini, or with Harris' hematoxylin and eosin stain.

The diameters of the secretory ducts in the Mallory's stained sections were determined by a micrometer placed inside the ocular of the microscope. The greatest length of the duct and the greatest width perpendicular to this length were determined. The average of these 2 values was used as the mean diameter of the duct. Approximately 200 measurements were made in each of the 4 groups. All measurements for each group were averaged and these results subjected to a statistical analysis.

Received for publication July 9, 1956.

*Hormone Assay Laboratories, Chicago, Ill.

RESULTS

Submandibular Gland.—Histologic examination of the glands removed from the hypophysectomized animals which had received no hormone injections revealed pronounced atrophic changes, most evident in the intralobular duct system. The epithelial cells composing the intralobular ducts contained a small amount of cytoplasm and no secretory granules were present. The nuclei of these cells had a somewhat vesicular appearance although they were not pycnotic. No basal striations in the duct cells were observed and the epithelium of the duct itself was of a low cuboidal type.

The submandibular glands of the group which had been injected with cortisone (Group 1) had intralobular ducts with a cuboidal epithelium. The duct cells themselves had a normal amount of cytoplasm but only in a few cells were any secretory granules present. The nuclei were located somewhat centrally and had an atypical, vesicular appearance resembling those seen in the hypophysectomized animals which had received no hormone injections. The most striking feature of this group was the presence of prominent, well-defined basal striations in the cytoplasm of the duct cells. Although the cortisone injections had no apparent effect upon duct size, the cells themselves were more distinct in appearance and had a greater amount of cytoplasm than those found in the uninjected hypophysectomized rats.

The injection of testosterone and thyroxine (Group 2) resulted in a marked increase in the number and size of the intralobular ducts as compared to those in both the hypophysectomized and cortisone-injected groups, and the intralobular ducts had a tall columnar epithelium, while the epithelial cells of these ducts were filled with large secretory granules. These granules were of such a size and number as to have crowded the nuclei away from the lumen of the tubule. The nuclei themselves were normal in appearance, usually with 2 nucleoli. There were no apparent basal striations since the granules themselves occupied almost the entire cell. It was obvious that in this group there was an hypertrophy of the intralobular duct system, evidently due to an increase in cell height and not to an increase in the size of the lumen, since the latter appeared unvarying in size. This hypertrophy seemed to be associated with an increased function of the gland, since many ducts were observed in which the secretory granules of the epithelial cells could be seen in the lumen of the duct.

The remaining group which had been injected with all 3 hormones (Group 3) had intralobular ducts with a tall columnar, secretory-type epithelium. The epithelial cells had basally located nuclei which were normal in appearance and which resembled those seen in the testosterone-thyroxine injected group. The cytoplasm of some of the cells had secretory granules, but fewer cells appeared to contain them and the granules were noticeably smaller than in the testosterone-thyroxine group. As in the cortisone-injected animals, basal striations were a prominent feature of the duct cells and these striations were readily apparent even in the few cells which had a large amount of granules in the cytoplasm. The duct hypertrophy which was so

marked in the testosterone-thyroxine group was also evident, but in this instance it was not always associated with an increased amount of secretory granules in the duct cells.

The data in Table I summarize the measurements on the diameters of the intralobular ducts of the 4 different groups. The injection of testosterone and thyroxine significantly increased the duct size ($p = .001$). Cortisone appeared to have no significant effect on duct size although the duct diameter was slightly smaller in the group receiving cortisone injections than in the control hypophysectomized animals. The injection of the 3 hormones together (testosterone, thyroxine, and cortisone) resulted in about the same increase in duct size that was found when testosterone and thyroxine were injected together. It was interesting to note that here again cortisone seemed to have a slight inhibitory effect, and a statistical comparison of the intralobular duct size of the testosterone-thyroxine and testosterone-thyroxine-cortisone injected groups revealed a smaller duct size in the latter animals, although it was of questionable significance ($p = .06$).

TABLE I
THE EFFECT OF THYROXINE, TESTOSTERONE, AND CORTISONE ON THE SIZE OF THE INTRALOBULAR DUCTS OF THE SUBMANDIBULAR GLAND IN THE HYPOPHYSECTOMIZED RAT

	CONTROL	THYROXINE AND TESTOSTERONE	CORTISONE	THYROXINE TESTOSTERONE CORTISONE
Final body weight (Gm.)	103	110	76	74
Mean diameter of serous tubule (microns)	35.9	44.6	34.8	43.3
Per cent difference compared to controls	—	+19.3	-3.0	+17.1
Statistical analysis p value	—	.001	0.2	.001

Parotid Gland.—The uninjected hypophysectomized animals had parotid glands which were somewhat atypical in appearance. The serous cells had indistinct outlines with scanty cytoplasm and a complete absence of any serozymogenic granules. The nuclei were pycnotic in appearance and irregular in shape. On the other hand, the animals receiving testosterone and thyroxine had normal appearing glands. The serous cells in this group had many fine secretory granules in an abundant cytoplasm, and their nuclei were normal in appearance.

The cortisone-injected group also had fine secretory granules in the serous acini, but they appeared to be fewer in number than those observed in the testosterone-thyroxine animals. The nuclei were pycnotic and closely resembled those of the uninjected hypophysectomized animals. The parotid glands of the group receiving all 3 hormones were essentially identical in appearance to those of the group which had been injected with testosterone and thyroxine.

DISCUSSION

Testosterone and thyroxine injections produce an hypertrophy of the intralobular duct system of the submandibular gland as well as an increase

in the number and size of their secretory granules. These data confirm those of other investigators.[1-3] Cortisone injections, however, appeared to have an inhibitory effect on the duct size and also the appearance of secretory granules, since the animals which had received cortisone in addition to testosterone and thyroxine had only a small number of cells with secretory granules as compared to the testosterone-thyroxine group. Baker[5] reported that the continuous injection of 3 to 6 mg. daily of ACTH produced hypertrophy of the intralobular ducts of the submandibular gland and an acinar atrophy of the parotid glands. However, he also pointed out that his ACTH preparation was probably contaminated with traces of ICSH, which could result in an increased secretion of testicular androgens. This might explain the observed duct hypertrophy. The data presented here indicate that, in the absence of the pituitary gland, cortisone has no stimulating effect and possibly even an inhibitory action on the rat submandibular gland. Shafer, Clark, and Muhler[7] have shown that cortisone does have some effect in preventing the precipitous fall in the submandibular gland proteolytic activity following hypophysectomy. However, compared to testosterone and thyroxine in combination, cortisone was ineffective in maintaining normal proteolytic activity.

The parotid atrophy resulting from ACTH injection reported by Baker was not produced in these experiments by cortisone injections, and it appeared that the only effect of cortisone upon the acini of the parotid gland was to increase the number of serozymogenic granules. These data emphasize that additional work is necessary before any interpretation of a possible interaction between the salivary glands and the adrenal glands may be made with definite clarity.

SUMMARY

The effect of hypophysectomy upon the histology of the rat salivary glands was studied. Also, the ability of testes, thyroid, and adrenal hormones to prevent hypophysectomy-induced changes was determined.

Hypophysectomy produced an atrophy of the duct system of the submandibular gland and a complete disappearance of secretory granules in the acini of both glands. The simultaneous administration of testosterone and thyroxine was effective in preventing hypophysectomy-induced changes in the parotid gland as well as the submandibular gland. However, cortisone by itself appeared to have no significant effect upon the histology of the salivary glands. When administered with testosterone and thyroxine, cortisone had a slight inhibitory effect upon intralobular duct size in the submandibular gland and the appearance of secretory granules in both parotid and submandibular glands. These results indicate that cortisone is not important in maintaining normal salivary gland histology and may even have an inhibitory effect when administered with testosterone and thyroxine.

REFERENCES

1. Shafer, W. G., and Muhler, J. C.: Effect of Gonadectomy and Sex Hormones on the Structure of the Rat Salivary Glands, *J. D. Res.* **32**: 262, 1953.

2. Grad, B., and Leblond, C. P.: The Necessity of Testes and Thyroid Hormones for the Maintenance of the Serous Tubules of the Submaxillary Gland in the Male Rat, *Endocrinology* **45**: 250, 1949.
3. Raynaud, J.: Action of Thyroxine on Submaxillary Gland of the Castrated Mouse, *Compt. rend. Soc. biol.* **144**: 245, 1950.
4. Raynaud, J.: Effect of Castration and Adrenalectomy Upon the Rat Submaxillary Gland, *Ann. d'endocrinol.* **8**: 359, 1947.
5. Baker, B. L.: A Comparison of the Histological Changes Induced by Experimental Hyperadrenocorticalism and Inanition, *Recent Prog. Hormone Res.* **7**: 331, 1954.
6. Shafer, W. G., and Muhler, J. C.: Experimental Dental Caries. VI. The Effect of Hypophysectomy on Dental Caries and the Salivary Glands of the Rat, *J. D. Res.* **34**: 531, 1955.
7. Shafer, W. G., Clark, P. G., and Muhler, J. C.: The Inhibition of Hypophysectomy-Induced Changes in the Rat Submaxillary Glands, *Endocrinology* **59**: 516, 1956.

THE RELATIONSHIP BETWEEN THE HISTOLOGY OF THE THYROID AND THE SALIVARY GLANDS AND THE INCIDENCE OF DENTAL CARIES IN THE RAT

DAVID BIXLER, PH.D., JOSEPH C. MUHLER, D.D.S., PH.D., AND WILLIAM G. SHAFER, D.D.S., M.S.

Indiana University School of Dentistry, Bloomington and Indianapolis, Ind.

AN INVERSE relationship appears to exist between the activity of the thyroid gland and the incidence of dental caries in the rat.[1-4] Thus, the feeding of dessicated thyroid,[1, 2, 4] or the injection of thyroxine,[5] significantly reduces dental caries, and the administration of antithyroid compounds, such as thiouracil,[1, 2, 4] or radioactive iodine,[3, 4] significantly increases dental caries. Five hundred microcuries of I^{131} in one dose significantly increased dental caries and was sufficient to inactivate completely the secretory activity of the thyroid gland.[3] However, it was also found that a total of 500 μc of I^{131} administered, either as 2 equally spaced injections of 250 μc, or 5 equally spaced injections of 100 μc each, was without significant effect on the dental caries experience although a marked atrophy of the thyroid gland was apparent as a result of both series of injections. The purpose of this experiment was to investigate the effect of a single injection of various graded doses of I^{131} upon the thyroid gland and dental caries in order to establish, if possible, what level of thyroid inactivity is correlated with histologically apparent alterations in the salivary glands and the incidence of dental caries.

EXPERIMENTAL

A total of 125 weanling male Sprague-Dawley rats was equally divided as to initial body weight into 5 experimental groups. Group 1 was injected intraperitoneally with 100 μc of radioactive, carrier-free I^{131} as the sodium salt. Groups 2, 3, and 4 received single intraperitoneal injections of 250, 500, and 750 μc, respectively, of I^{131}. The remaining group received no I^{131} and served as a control. All animals were fed ad libitum a coarse corn cariogenic diet[6] and distilled water for the experimental period of 140 days. The animals were housed in pairs in raised screen cages in a temperature-controlled room. At the termination of the experimental period the animals were sacrificed with ether and the heads removed for dental caries evaluation.[7] The thyroid and salivary glands were removed, fixed in Bouin's solution, and examined histologically.

The diameter of the granular tubule of the submaxillary glands of 5 rats in each group was determined with the aid of a micrometer eyepiece. All measurements for each group were averaged and these results subjected to statistical analysis.

Received for publication July 12, 1956.

RESULTS

Graded doses of I^{131} produced a graded response in dental caries (Table I). Although 100 μc of I^{131} was without a significant effect, each higher level of I^{131} produced a higher caries incidence, with the group receiving 750 μc showing the highest experience. Similarly, as the dosage of I^{131} was increased, an increase was also noted in both the number of molars affected and the severity of the carious lesions.

TABLE I
EFFECT OF GRADED DOSES OF I^{131} ON DENTAL CARIES IN THE RAT

I^{131} ADMINISTERED (μC)	NO. ANIMALS	FINAL BODY WT. (GM.)	MEAN NO. OF LESIONS	MOLARS AFFECTED	SEVERITY*
100	23	377	7.4	3.9	2.6
250	21	366	9.2	4.4	2.7
500	19	239	9.6	4.8	2.8
750	17	194	10.4	4.9	2.9
Control	26	361	7.3	3.6	2.3

*Based on the following system: Tooth 0 to ¼ destroyed—1; tooth ¼ to ½ destroyed—2; and tooth ½ to completely destroyed—3.

Thyroid Gland.—The thyroid glands from both the control and 100 μc groups were essentially the same, although in the latter group the glands appeared to have a slightly increased amount of connective tissue (Table II). The nuclei were normal and the epithelium of the follicles varied from columnar to low cuboidal. In the larger follicles the colloid was frequently observed to be vacuolated. The parathyroid glands were normal in appearance in both groups.

TABLE II
EFFECT OF GRADED DOSES OF I^{131} UPON THE HISTOLOGY OF THE THYROID GLAND

I^{131} ADMINISTERED (μC)	NO. THYROID GLANDS	EPITHELIUM OF FOLLICLE	NUCLEAR APPEARANCE	AMOUNT OF CONNECTIVE TISSUE INVASION*	FUNCTIONAL ACTIVITY OF GLAND†
Control	6	Cuboidal to columnar	Normal	0	++++
100	6	Cuboidal to columnar	Normal	0 (+)	+++
250	6	Cuboidal	Normal and pyenotic	+	+
500	6	Low cuboidal	Pyenotic	++	0
750	6	Low cuboidal	Pyenotic	+++	0

*Plus signs indicate increasing amounts of connective tissue.
†Estimation based upon predominant type of epithelium, presence and appearance of colloid in follicle, amphoteric nature of colloid, and nuclear morphology.

The thyroid glands from the 250 μc group showed definite effects of the radiation. The follicles were reduced in size; approximately 50 per cent of them contained colloid and this appeared frothy and vacuolated. The nuclei were often normal appearing, although occasionally pyenotic nuclei associated with an atrophied follicle were observed. The epithelium of the majority of the follicles was cuboidal and a pronounced connective tissue invasion was apparent. The glands gave the appearance of having been almost entirely

destroyed by the initial dose of radioactive iodine, but subsequently had regenerated to a limited degree. Thus, a small amount of what appeared to be functional thyroid tissue was present in each gland examined, although the major portion of the gland was atrophic. The parathyroid glands appeared normal.

Both 500 μc and 750 μc of I^{131} appeared to have the same degree of destructive effect upon the thyroid gland. The glands themselves were mostly connective tissue and the few follicles which remained were atypical in appearance. The nuclei were pyenotic and the epithelium was low cuboidal. The thyroid glands of both these groups gave a striking picture of inactivity and atrophy. It was particularly difficult in the 750 μc group to find any thyroid tissue at all. Connective tissue composed almost the entire gland with only a few scattered, nonfunctional follicles present.

The parathyroid glands in these groups seemed to show some slight effect of the radiation, although the cell nuclei themselves were normal in appearance, and the over-all histologic picture was one of a normally functioning gland.

Submaxillary Gland.—Since previous reports have clearly indicated that the structure of the submaxillary gland is regulated at least in part by the thyroid gland,[8, 9] the effects cited here (Table III) are most likely the direct result of loss of thyroid function.

TABLE III
EFFECT OF GRADED DOSES OF I^{131} ON SIZE OF GRANULAR TUBULES OF SUBMAXILLARY GLAND

I^{131} ADMINISTERED (μC)	NO. SUBMAXILLARY GLANDS	NO. MEASUREMENTS	MEAN DIAMETER OF GRANULAR TUBULES	P VALUE†	TUBULES SHOWING GRANULES (%)
Control	5	100	54.9 ± 3.7*	–	98
100	5	100	51.5 ± 3.9	.007	57
250	5	100	43.3 ± 2.8	.001	22
500	5	100	35.8 ± 2.2	.001	0
750	5	100	30.1 ± 1.1	.001	0

*Standard deviation.
†Each mean diameter compared to that of the succeeding group rather than each one to the control.

The administration of 100 μc of radioiodine significantly reduced the diameter of the granular tubules but was without significant effect upon the other structural components of the gland. However, one striking alteration was noted in the cells composing the granular tubule. These cells ordinarily are filled with large, highly refractile granules. In the 100 μc group there was approximately a 40 per cent reduction in the number of tubules which contained these granules. This same group exhibited no significant alterations in the thyroid glands or in the incidence of dental caries.

The injection of 250 μc of I^{131} produced a significant reduction in tubule size in comparison to that observed following administration of 100 μc (Table III), and a further reduction in the number of tubules showing granules was observed. No other histologic changes were noted.

The administration of 500 μc and 750 μc of I^{131} produced nearly the same effect on the submaxillary gland, i.e., complete loss of granules from both the acini and the intralobular duct system, a significant reduction in tubule diameter, the appearance of vesicular atypical nuclei and, apparently, a marked retardation in the development of the duct system. Thus, the control and lower I^{131} concentration groups had many convoluted appearing tubules, whereas the higher I^{131} groups had uniformly small and oval-shaped tubules.

DISCUSSION

The results of this experiment show that the activity of the thyroid gland, as judged by histologic study, is closely related to the incidence of dental caries in the rat. The animals receiving a concentration of radioiodine (100 μc) which did not significantly alter the structure of the thyroid gland had a normal, expected incidence of dental caries but higher levels of I^{131}, which resulted in pronounced atrophic changes in the thyroid gland, were associated with significant increase in dental caries.

The histologic appearance of the submaxillary gland, particularly the tubular portion of the intralobular duct system and its granule content, was closely related to the activity of the thyroid gland. Large tubules and many granules in the tubule cells were found in the groups with normal caries indices (control and 100 μc I^{131}), whereas a marked reduction in size of the tubules and the absence of granules were found in groups with the highest caries indices (500 and 750 μc I^{131}). Shafer, Clark, and Muhler[10] and Sreebny[11] proposed that these granules represent sites of proteolytic activity, since their absence is associated with low activity and an abundance of these granules is associated with normal proteolytic activity. In addition, Shafer and Muhler[12] have proposed that an inverse relationship exists between the presence of the tubule granules and dental caries incidence. This study shows that dental caries incidence can be correlated with the size of the granular tubules and the presence of their granules. Since these same histologic structures in the submaxillary gland are shown to be closely related to the activity of the thyroid gland, as judged by histologic criteria, it seems a possibility that the effects of altered thyroid activity on dental caries[1-4] may actually be expressed through the activity of the salivary glands.

SUMMARY

The administration of single doses of 100, 250, 500, and 750 μc of radioactive iodine (I^{131}) to 4 groups of weanling rats produced a graded increase in their dental caries experience. Histologic study of the thyroid glands from the various groups revealed that 100 μc of I^{131} was without significant effect upon the thyroid gland, whereas each higher dosage of I^{131} produced a greater and more pronounced atrophy. Thus, a direct relationship between thyroid function and dental caries experience in the rat seems apparent. Furthermore, histologic study of the submaxillary glands of the animals in the various groups revealed a progressive nonfunctioning, atrophic appearance with increasing concentrations of radioactive iodine. From these results it appears that loss

of thyroid function may be intimately associated with loss of salivary gland function. The salivary glands then are considered as possible intermediates in a thyroid gland–dental caries relationship in the rat.

REFERENCES

1. Muhler, J. C. and Shafer, W. G.: Experimental Dental Caries IV. The Effect of Feeding Desiccated Thyroid and Thiouracil on Dental Caries in Rats, *Science* **119**: 687, 1954.
2. *Idem:* Experimental Dental Caries VIII. Effect of Thyroid-Testosterone Administration on Dental Caries in Rat, *Proc. Soc. Exper. Biol. & Med.* **88**: 191, 1955.
3. Bixler, D., Muhler, J. C., and Shafer, W. G.: The Effect of Radioactive Iodine on Dental Caries in the Rat, *J. A. D. A.* **53**: 667, 1956.
4. Muhler, J. C., and Bixler, D.: The Relation of Systemic Fluoride and Thyroid Gland Activity to the Incidence of Dental Caries in the Rat, *J. D. Res.* **36**: 304, 1957.
5. Bixler, D., Muhler, J. C., and Shafer, W. G.: Unpublished data.
6. Muhler, J. C. and Day, H. G.: Effects of Stannous Fluoride, Stannous Chloride and Sodium Fluoride on the Incidence of Dental Lesions in Rats Fed a Caries-Producing Diet, *J. A. D. A.* **41**: 528, 1950.
7. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Comparison of Different Preparations of Stannous Fluoride With Sodium Fluoride in the Prevention of Dental Caries in the Rat, *J. A. D. A.* **46**: 290, 1953.
8. Grad, B. and LeBlond, C. P.: The Necessity of Testes and Thyroid Hormones for the Maintenance of the Serous Tubules of the Submaxillary Gland in the Male Rat, *Endocrinology* **45**: 250, 1949.
9. Raynaud, J.: Action of Thyroxine on Submaxillary Gland of the Castrated Mouse, *Compt. rend. Soc. biol.* **134**: 223, 1950.
10. Shafer, W. G., Clark, W. G., and Muhler, J. C.: The Inhibition of Hypophysectomy-Induced Changes in the Rat Submaxillary Gland, *Endocrinology* **59**: 516, 1956.
11. Sreebny, L. M.: Histologic and Enzymatic Differences in the Submaxillary Glands of Normal and Hypophysectomized Male and Female White Rats, *J. D. Res.* **32**: 686, 1953.
12. Shafer, W. G., and Muhler, J. C.: Experimental Dental Caries VI. The Effect of Hypophysectomy on Dental Caries and the Salivary Glands of the Rat, *J. D. Res.* **34**: 531, 1955.

ENLARGEMENT OF SUBMANDIBULAR SALIVARY GLANDS OF RATS RESTRICTED TO ACIDIC AND BASIC SOLUTIONS AS THE SOLE SOURCE OF DRINKING FLUID

GERALD J. COX, WILLARD G. CLEMENTS, EDWARD C. HILGER, JR., AND DAVID W. DEGRANGE

Research Department, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.

IN THE COURSE of studies of the relative effects of various acids in the drinking fluid on the etching of the molar teeth of rats and possible relation of such etching to (a) initiation and (b) promotion of the carious process, the submandibular glands of the animals were excised and weighed as a test of possible enlargement of the glands through repeated stimulation by the sourness of the acids. In 11 littermate groups of 5, with 1 rat in each group being on water, in no case was the largest submandibular gland found in the rats on water.[1] On the basis of this evidence of enlargement of salivary glands by acid stimulation, further studies were initiated to determine the relative effectiveness of unbuffered solutions of different hydrogen ion concentrations in enlargement of the submandibular salivary glands.

MATERIALS AND METHODS

Rats, of the Long-Evans strain,* derived from stock maintained on Ration 36 (Table I) were continued on that ration for 4 weeks after weaning at 21 days of age (experimental period). Water, and other drinking fluids containing the substances shown in Table II at 0.05 molar concentration, were provided, ad libitum, during the experimental period, in 6-ounce glass bottles with a glass drinking tip.[2] The studies were made during seasons requiring heating of the animal room and hence there was no significant variation of environmental temperature and humidity to cause varying intake of water in successive studies. After 4 weeks on the experimental fluids all animals were killed by chloroform vapors and the carcasses weighed to the nearest gram. The submandibular glands were immediately dissected and weighed individually on a Roller-Smith precision balance to the nearest 0.2 mg. The ratios of the mean weight of each pair of glands to body weight were calculated on a per mille basis.

Presented at the Thirty-fourth General Meeting of the International Association for Dental Research at St. Louis, Mo., March 22, 1956. This investigation was supported by a Research Grant D-191-C from the National Institute of Dental Research of the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

Received for publication July 21, 1956.

*Original stock purchased from Pacific Animals Farms, Los Angeles 39, Calif.

TABLE I

COMPOSITION OF RATION 36 USED DURING PREGNANCY, LACTATION, AND THE EXPERIMENTAL PERIOD

		PER CENT
Whole ground wheat	100 lbs.	72.4
Whole milk powder	20 lbs.	14.5
Lactalbumin	10 lbs.	7.3
Alfalfa meal	4 lbs.	2.9
Sodium chloride	2 lbs.	1.5
Calcium carbonate	550 Gm.	0.9
Cod liver oil	300 Gm.	0.5

In Table II are shown the data of (a) mean weights of the rats at start on acids, (b) mean weights at sacrifice, (c) mean weight gains in 4 weeks, and (d) range of the gland to body weight ratios.

TABLE II

DISTRIBUTION OF RATS TO VARIOUS DRINKING FLUIDS, WEIGHT GAINS, AND RATIOS OF GLAND TO BODY WEIGHT

	NUMBER OF RATS		MEAN WEIGHTS, GM.				GLAND/BODY WEIGHT, RANGE*
			ALL	SURVIVORS			
FLUID, pH	AT START	SURVIVORS	AT START	AT START	AT END	GAINS	
$NaHCO_3$ 8.7	23	20	34.7	34.2	106.8	72.6	1.18-1.57
Water	32	28	34.5	35.4	103.2	67.8	0.97-1.51
Acetic 3.0	32	29	32.5	33.3	96.5	63.2	0.96-1.94
Lactic 2.5	18	18	32.1	32.1	97.1	65.1	1.25-1.76
Citric 2.3	30	27	33.0	35.2	97.4	62.5	1.10-2.05
Tartaric 2.2	33	27	32.4	34.1	80.7	46.6	1.19-2.07
H_3PO_4 1.7	32	28	32.3	33.6	85.3	51.8	1.25-2.14
Oxalic 1.6	32	27	31.3	33.4	85.0	51.6	0.95-1.73
Totals	232	204					

*Per mille.

Since the critical values upon which decisions were to be made with respect to gland size were ratios and thus were not in true arithmetic series, the data have been analyzed by the method of Cox, Dodds, Dixon, and Matuschak.[3] This procedure is based only on relative magnitude of the ratios. In such analysis, quantitative relations of magnitude of difference of ratios is lost, since the ratios are evaluated simply as greater than or less than. The method of analysis is hence conservative.*

*The formula $t = \frac{m-b}{\sqrt{npq}}$, used for analysis of data of a binominal distribution, reduces to $t = \frac{a-b}{\sqrt{a+b}}$ in which "a" and "b" are frequencies of occurrence of two related variates with a priori probability of 0.5.

The data of gland to body weight ratios of rats on the various acids, in comparison with those of littermates of the same sex on water, are shown in Table III, together with the "t" values derived by the method of Cox, Dodds, Dixon, and Matuschak.[3] Included are the odds against chance occurrence of such a distribution of ratios as observed.

TABLE III

COMPARISON OF GLAND-BODY WEIGHT RATIOS OF RATS ON WATER VS. THOSE ON OTHER FLUIDS

FLUID, 0.05M	GLANDS LARGER		"t" VALUE	ODDS AGAINST CHANCE
	WATER	FLUID		
$NaHCO_3$	7	6	0.3	1.3
Acetic	6	15	2.0	20
Lactic	1	12	3.1	437
Citric	3	20	3.5	2,500
Tartaric	1	18	3.9	10,395
H_3PO_4	2	18	3.6	2,910
Oxalic	11	7	0.9	2.9

In Table IV are shown the generalized data of intercomparison of all possible pairings, together with the "t" values. The derived values of odds against chance occurrence are not given in Table IV. Decision of statistical signficance has been made on the basis of "t" exceeding 1.96 or odds exceeding 19 to 1.

TABLE IV

FREQUENCIES* OF GLAND-BODY WEIGHT RATIOS GREATER ON ONE OF TWO DRINKING FLUIDS AND "t" VALUES OF THE DISTRIBUTION

FLUID, pH	$NaHCO_3$ 8.7	WATER	ACETIC 3.0	LACTIC 2.5	CITRIC 2.3	TARTARIC 2.2	H_3PO_4 1.7
Water	6 7 0.3						
Acetic 3.0	6 6 0	6 15 2.0					
Lactic 2.5	1 5 1.6	1 12 3.1	5 12 1.7				
Citric 2.3	2 12 2.7	3 20 3.5	6 15 2.0	4 11 1.8			
Tartaric 2.2	1 9 2.5	1 18 3.9	8 13 1.1	3 12 2.3	7 11 0.9		
H_3PO_4 1.7	2 10 2.3	2 18 3.6	9 14 1.0	5 10 1.3	10 10 0	10 13 0.6	
Oxalic 1.6	7 3 1.3	11 7 0.9	14 4 2.4	13 0 3.6	15 2 3.2	17 1 3.8	17 1 3.8

*The upper of the two vertically placed values is the number of rats with gland-body weight ratios greater for the fluid at the head of the column; the lower value is number of rats with ratios greater for the fluid listed at the left side of the table. The third number is the "t" value.

DISCUSSION

There are 2 reports of changes in size of salivary glands of rats under experimental conditions.

Schwartz and Shaw,[4] in a study of caries incidence in rats subsequent to selective desalivation, reported "moderate to large increases in the average absolute weight of the remaining major salivary glands when either the parotids, sublinguals, submaxillaries, parotids and submaxillaries, sublinguals and submaxillaries, or parotids and sublinguals were removed. The results indicate that under these experimental conditions a compensatory increase in size of the remaining major salivary glands does occur. Even when the small major sublingual glands alone were extirpated, the parotid glands increased in size."

Sreebny, Meyer and Bachem,[5] in a study of submandibular glands of the caries-susceptible and caries-resistant rats developed by Hunt, Hoppert, and Erwin[6] found using 8 rats of each strain, "The dry weight of the glands in the susceptible strain was slightly higher than the dry weight of the glands in the resistant strain and represented a higher fraction of the total body weight. The ratio of dry weight of gland over total body weight ranged from 21 to 25 parts in 100,000 in the susceptible animals and from 15 to 23 parts in the resistant animals, averaging 23 and 19, respectively."

In Table III is shown that there is no significant difference in gland to body weight ratios produced by sodium bicarbonate or oxalic acid, when present in the drinking fluid of rats at 0.05 molar concentration, in comparison with the ratios obtained from littermate rats receiving water. However, when acetic, lactic, citric, phosphoric, or tartaric acid is present in the drinking fluid at 0.05 molar concentration, significant increases of the gland to body weight ratios are produced.

In Table IV the fluids are listed in the order of decreasing pH values, as is also done in Tables II and III. The "t" values are shown for all possible comparisons in Table IV. In general, the lower the pH of the drinking fluid the more likely are the submandibular salivary glands of the rat to be increased in size.

In Tables III and IV it is evident that oxalic acid in the drinking fluid does not result in increased gland to body weight ratios in comparison with water and this anomalous effect is clearly brought out by comparison with the other acids. The tendency is possibly to diminish the ratio.

The data available from this study give no clues as to why oxalic acid fails to produce an increase in the gland to body weight ratios. The rats ingesting the tartaric acid solutions showed lower body weight gains in the 4-week experimental period than those of any other group but the gland to body weight ratios of this group are consistent with the findings related to order of pH of the other acids. It may be speculated that oxalic acid may have specific toxic effects related to development of the submandibular glands or there may be specific oral effects, such as inhibition of stimulation by the precipitation of calcium oxalate on nerve ends.

The data for acetic acid show a barely significantly different effect from water. However, by use of the comparisons with the other acidic solutions

it may be concluded that acetic acid is effective in increasing the gland to body weight ratio.

The highest of the "t" values resulted from tartaric and phosphoric acids in comparison with water and oxalic acid. With respect to water the effect may, in part, be related to the low rate of increase in body weight.

SUMMARY

The submandibular salivary glands of rats were significantly enlarged when the sole drinking fluid was unbuffered 0.05 molar acetic, lactic, citric, tartaric, or phosphoric acid, during the period 21 to 49 days of age, comparison being made with the glands of littermate rats with water to drink. No increase in size of the glands was caused by 0.05 molar sodium bicarbonate or oxalic acid.

REFERENCES

1. Clements, W. G., Hilger, E. G., Jr., DeGrange, D. W., and Cox, G. J.: Weight Study of the Submandibular Salivary Glands of Acid-Fed Rats, Proceedings of I.A.D.R., Abstract 12, 1956.
2. Morgan, P. V., and Cox, G. J.: Devices for Small Animal Rooms, *Animal Care Panel, Fourth Ann. Meeting*: 192-193, 1953.
3. Cox, G. J., Dodds, M. L., Dixon, S. F., and Matuschak, M. C.: Experimental Dental Caries. III. A System of Recording Corn Meal Caries in Rats, *J. D. Res.* **18**: 469-480, 1939.
4. Schwartz, A., and Shaw, J. H.: Studies on the Effect of Selective Desalivation on the Dental Caries Incidence of Albino Rats, *J. D. Res.* **34**: 239-247, 1955.
5. Sreebny, L. M., Meyer, J., and Bachem, E.: Morphologic and Enzymatic Differences in Caries-Resistant and Caries-Susceptible Albino Rats, *J. A. D. A.* **53**: 9-13, 1956.
6. Hunt, H. R., Hoppert, C. A., and Erwin, W. G.: Inheritance of Susceptibility to Caries in Albino Rats (Mus Norvegicus), *J. D. Res.* **23**: 385-401, 1944.

OXIDATIVE ENZYMES

I. Succinic Dehydrogenase, DPN Cytochrome *c* Reductase, Cytochrome Oxidase, and Catalase in Oral, Liver, and Brain Cortex Tissues

BERTRAM EICHEL* AND ARNOLD A. SWANSON

Departments of Experimental Dentistry and Pathology, USAF School of Aviation Medicine, Randolph Air Force Base, Texas

With the Technical Assistance of Airman Third Class Tommie Bradley

MUCH attention has been devoted to various intracellular oxidative enzymes in such tissues as the liver, brain, heart, and kidney; whereas, little attention has been directed toward those tissues which make up the mouth and eye.

Submaxillary gland tissue slices have been evaluated manometrically[1] and by the Thunberg method[2] for succinic dehydrogenase activity. Succinic dehydrogenase activity has also been studied in extracts of human tooth pulp using the Thunberg method.[3] Rat submaxillary gland cytochrome oxidase has been estimated manometrically,[4] and the enzyme has been studied in mouse tissue slices employing the Nadi reagent.[2] Succinoxidase, the succinic dehydrogenase-cytochrome *c*–cytochrome oxidase complex, has been estimated manometrically in rat submaxillary gland.[4] To our knowledge, DPN† cytochrome *c* reductase and catalase have not been previously studied in mammalian oral tissues.

In this report, tooth pulp, submaxillary gland, gingiva, and tongue mucosa of normal rabbits were selected for evaluation. The retina, choroid and crystalline lens were also studied.[5] The liver and brain cortex‡ were included as controls. Succinic dehydrogenase, DPN cytochrome *c* reductase, cytochrome oxidase and catalase[6, 7] have been quantitatively estimated by spectrophotometric analysis in each of the above-named tissues. Comparisons between the mean levels of each tissue unit enzyme activity has been made by arbitrarily assigning a value of 100 per cent to the mean unit enzyme activities detected in liver. Total protein analyses were also performed upon each tissue.[6, 7]

EXPERIMENTAL

Animals and Diets.—In the first experiment reported here, normal virgin doe New Zealand white and chinchilla gray rabbits, approximately 6 months old, between 6 and 7 pounds, were maintained on a stock rabbit chow and water diet, fed ad libitum. Only New Zealand white rabbits were used in the second experiment.

This work was supported under USAF School of Aviation Medicine Task No. 757-010.
Received for publication Feb. 13, 1956; revised by authors March 27, 1957.

*Present address: Department of Biochemistry, Jewish Hospital of Brooklyn, 555 Prospect Place, Brooklyn, N. Y.

†Diphosphopyridinenucleotide (coenzyme 1).

‡Cerebral gray matter.

Handling of Tissues.—The rabbits were sacrificed by decapitation. The tooth pulp from the two upper first incisors (used in experiment I) and from the two lower first incisors (experiment II), the tongue mucosa (right half of the anterior-superior area stripped free of muscle tissue), and the total buccal gingival tissue of the right and left molar mandibular regions were dissected out of the oral cavity. Approximately 100 to 150 mg. of liver (periphery of lobe), brain cortex (gray matter from right occipital area), and submaxillary gland tissues were also taken for study. Approximately 10 minutes were required for the removal of the tissue samples from a given animal. The tissue wet weights were detemined with a Mettler Gramatic balance, and were immediately placed in small Ten Broeck glass homogenizers and homogenized with 0.5 ml. ice cold 0.05 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.4. These homogenates were further diluted and rehomogenized with ice cold buffer to yield a 1:50 (weight/volume) final dilution.

Microprotein Method.—A modification* of the method of Kabat and Mayer[8] was used. Essentially 2 reagents are required: dilute Folin-Ciocalteau phenol reagent (1.0 volume of reagent plus 2.0 volumes of H_2O), and the protein reagent (100 ml. of 4 per cent Na_2CO_3 plus 1.0 ml. of 2 per cent $CuSO_4 \cdot 5H_2O$ plus 1.0 ml. of 4.9 per cent sodium-potassium tartrate). Varying concentrations of bovine serum albumin (50-200 γ of protein) were routinely used to provide a standard curve each day.

The components of the reaction mixture were added in the following order: the standard protein or tissue homogenate, sufficient H_2O to yield a final volume of 3.0 ml., 2.0 ml. of protein reagent (incubate for 10 minutes at 45° C.) and 0.2 ml. of Folin reagent (incubate for 15 minutes at room temperature) and read at 700 mμ in the Beckman spectrophotometer.

Enzyme analyses were performed with Beckman Model DU spectrophotometers. Each instrument was fitted with thermospacers and an assembly block in which water circulated from a constant temperature bath maintained at 29.8° C. All reactants were kept in test tubes in the water bath throughout the experiment. The tissue homogenates, in the original homogenizer tubes, and the (red) DPN and TPN† solutions, in test tubes, were stored in a cracked ice bath throughout the experiment. The enzyme assays for succinic dehydrogenase,[9] DPN cytochrome *c* reductase,[10] cytochrome oxidase,[11] and catalase,[12] were modified slightly. The concentrations and order of addition of components into the reaction cuvette for each enzyme assay are given in Figs. 3 through 9. Fresh solutions of each reagent were prepared daily with the exception of sodium succinate and nicotinamide which were prepared twice weekly and stored at –15° C. when not in use.

Single enzyme determinations were performed with each tissue. Zero time was taken as the time of inversion of the covered cuvette immediately following the addition of the last reactant. The first density reading and time in seconds

*The authors wish to thank Dr. S. J. Cooperstein for his modification of this method, given by personal communication.

†(Red) DPN is reduced diphosphopyridinenucleotide (reduced coenzyme 1) and TPN is triphosphopyridinenucleotide (coenzyme 2).

was recorded as soon as possible. Subsequent time intervals were taken at densities 0.025, 0.010, or 0.005 below or above the initial one in accord with the given reaction under study. Generally no reaction was recorded longer than a 10-minute period. Most of the reactions were designed to yield a minimum of 8 readings, at the density intervals sighted, within a 2- to 5-minute period. Density was plotted against time in seconds for each tissue reaction studied. The slope of the resultant line yielded the velocity constant.

Reaction kinetics: Succinic dehydrogenase and DPN cytochrome *c* reductase behaved in accord with zero order reaction kinetics with respect to the change in concentration of cytochrome *c* and DPN, respectively; whereas cytochrome oxidase and catalase obeyed first order reaction kinetics with respect to the change in concentration of cytochrome *c* and H_2O_2, respectively. The specific or unit enzyme activities (Figs. 3 through 9) are quantitative measures of the tissue enzyme concentrations and are expressed as the velocity constant (V) per milligram of protein for each tissue homogenate under the conditions of each enzyme assay.

The velocity constants for the zero order reactions were determined by

Formula (1): $$V = \frac{D_t - D_o}{(\alpha \text{ red.} - \alpha \text{ oxid.})\ T}$$

and for the first order reactions by Formula (2):

$$V = \frac{1}{T} \ln \frac{D_r}{D_r - (D_o - D_t)}.$$

Where V is the velocity constant (for succinic dehydrogenase and DPN cytochrome *c* reductase V is Δ ferricytochrome *c*, for cytochrome oxidase V is Δ ferrocytochrome *c*, and for catalase V is Δ H_2O_2), D_t is the density at any time on the curve, D_o is the density at zero time (obtained by extrapolation to the y axis), D_r is the difference in the density of the reduced and oxidized forms of the substance whose change is followed, α red. and α oxid. are the molar extinction coefficients of the reduced and oxidized forms of the substance whose change is followed (α red. cytochrome $c = 2.64 \times 10^7$ cm.^{2}/mole, Tint and Reiss,[13] and α oxid. cytochrome $c = 0.90 \times 10^7$ cm.^{2}/mole, Umbreit, Burris, and Stauffer[14]) T is the time in seconds, and ln is the natural log.

RESULTS

Two distinct studies (experiments I and II) on normal rabbit tissues have been correlated in this report. Figs. 1 through 9 show the experimental values obtained. The mean values for each tissue analysis are represented by bars, New Zealand white rabbits (white bar) and chinchilla gray rabbits (diagonal line bar).

The classification in numerical sequence of the several tissues into groups in accord with their total protein or unit homogenate enzyme activity content was found to be significantly different at the 5 per cent probability level or lower following statistical analysis, e.g., Group 2 compared to Group 1.

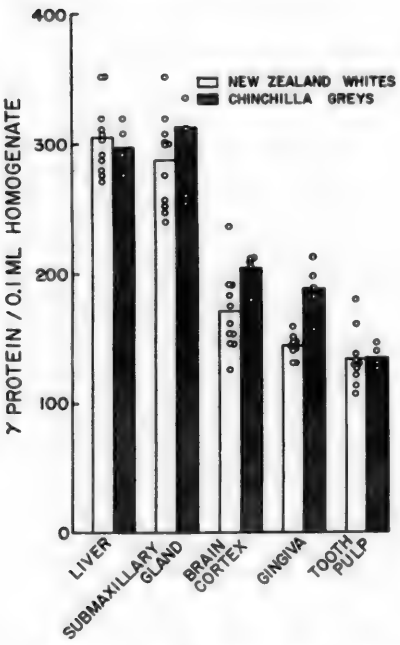


Fig. 1.—Unit total protein of normal rabbit tissue homogenates, experiment I. The following volumes of each tissue homogenate (1:50) dilution were used: 0.25 ml. of liver and submaxillary gland, 0.05 ml. of brain cortex, tooth pulp, and gingiva.

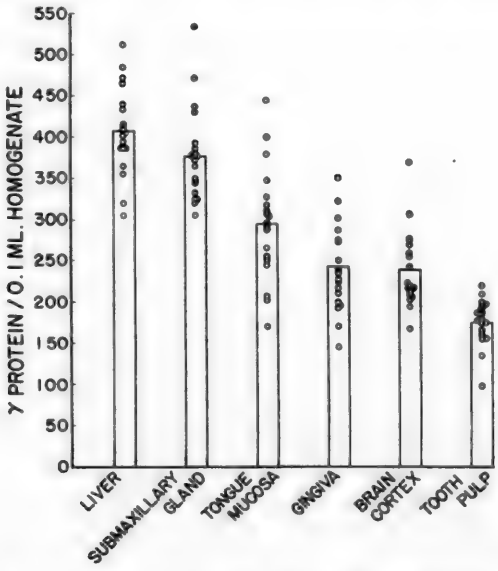


Fig. 2.—Unit total protein of normal rabbit tissue homogenates, experiment II. The same amounts of homogenate as indicated in Fig. 1 were used—in addition 0.05 ml. of the tongue mucosa homogenate was taken for assay.

Further inquiry into the relative levels of each component was made by comparing each tissue with the liver (assigning a value of 100 per cent to the mean liver values, Tables I and II).

TABLE I
PER CENT UNIT PROTEIN COMPARED TO LIVER

TISSUE	EXPERIMENT I		EXPERIMENT II
	NZW* (%)	CG† (%)	NZW* (%)
Liver	100	100	100
Submaxillary gland	94	105	92
Brain cortex	56	69	58
Tongue mucosa	—	—	72
Tooth pulp	44	45	43
Gingiva	47	63	59

*New Zealand white rabbits.
†Chinchilla gray rabbits.

TABLE II
PER CENT UNIT ENZYME ACTIVITY COMPARED TO LIVER

TISSUE	SUCCINIC DEHYDROGENASE			DPN CYTOCHROME *c* REDUCTASE			CYTOCHROME OXIDASE			CATALASE		
	EXP. I		EXP. II	EXP. I		EXP. II	EXP. I		EXP. II	EXP. I		EXP. II
	NZW* (%)	CG† (%)	NZW (%)	NZW (%)	CG (%)	NZW (%)	NZW (%)	CG (%)	NZW (%)	NZW (%)	CG (%)	NZW (%)
Liver	100	100	100	100	100	100	100	100	100	100	100	—
Submaxillary gland	109	94	158	122	88	79	121	109	153	16	17	—
Brain cortex	84	65	117	175	132	—	182	159	—	8	6	—
Tongue mucosa	—	—	71	—	—	20	—	—	59	—	—	—
Tooth pulp	49	40	31	19	18	10	20	24	12	23	18	—
Gingiva	31	29	13	16	11	4	16	29	9	24	16	—

*New Zealand white rabbits.
†Chinchilla gray rabbits.

Total protein analyses were performed in duplicate upon aliquot samples of each tissue homogenate. The data collected for experiment I are given in Fig. 1. Inspection of the mean values or the grouping of the individual values shows that the tissues studied may be classed into two general groups based upon their protein content. Liver and submaxillary gland contained about equal concentrations of protein. Brain cortex, gingiva, and tooth pulp contained the lesser amount of protein.

Reasonably good duplication of results are evident when the chinchilla gray rabbit data are compared to the New Zealand white rabbit data even though fewer chinchilla gray rabbits were studied. Table I summarizes the mean protein levels in terms of per cent protein of wet weight for each tissue as compared to liver.

The data of experiment II are summarized in Fig. 2. In this series, tongue mucosa, as well as the other oral tissues, was studied. The tissues may be classed into four groups based upon this series. Liver and submaxillary gland make up Group 1, and contain the highest mean total protein. Tongue mucosa constitutes Group 2, gingiva and brain cortex make up Group 3, and the tooth pulp falls into Group 4. The latter tissue yielded the lowest mean total protein content.

The mean protein levels expressed in terms of per cent protein of wet weight for each tissue, as compared to liver, are also summarized in Table I. The tissue relationships established in experiment I are confirmed, with the exception that the value for the New Zealand white rabbit gingiva is higher in the second experiment than in the first. Confirmation of the protein concentration found in the gingiva of the chinchilla gray rabbits, however, emerges.

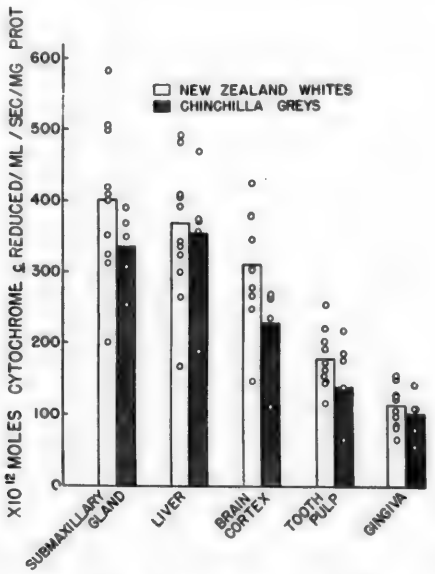


Fig. 3.—Succinic dehydrogenase unit activities of normal rabbit tissue homogenates, experiment I. The reaction mixtures contained in the cuvettes were added in the following concentrations and sequence: 0.025 ml. of liver and submaxillary gland, 0.05 ml. of brain cortex and 0.10 ml. of gingiva and tooth pulp tissue homogenates; 0.10 ml. of 0.5 M sodium succinate (dissolved in 0.15 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.4); 1.00 ml. of 0.15 M KH_2PO_4-Na_2HPO_4 buffer (pH 7.4 when diluted 1:3 times with H_2O); sufficient distilled H_2O to yield a total reaction volume of 3.0 ml.; 0.10 ml. of 3.0×10^{-2}M NaCN; 1.00 ml. ferricytochrome c* (0.5 mg. of pure cytochrome *c* per 1.00 ml. of H_2O). The reduction of ferricytochrome C was followed at 550 mμ, with a slit width of 0.01 mm.

Tissue enzymes: Preliminary study of each enzyme yielded good straight line proportionality when reaction velocity was related to tissue homogenate concentration. The subsequent selection of each tissue homogenate volume used in experiments I and II was based upon these studies. The specific enzyme protein was the limiting factor in each assay, since the enzyme activities obtained always fell somewhere upon the tissue homogenate concentration—reaction velocity curves.

The following enzyme activities detected in each of the tissue homogenates studied may be regarded as a measure of the enzyme concentration or enzyme

*The cytochrome *c* used in experiment I was supplied, in the lyophylized state, by the Sigma Chemical Company, and that in experiment II, in solution, by Wyeth, Inc. The Wyeth protein was 3× dialyzed against Baxter sterile nonpyrogen distilled water and then lyophilized. Spectral characterization of the reduced preparations showed typical absorption peaks at 550 and 520 mμ. Using the molar extinction coefficient of Tint and Reiss for reduced beef heart cytochrome *c*, the calculated purity for the Sigma cytochrome *c* and the lyophilized Wyeth cytochrome *c* was found to be 57 per cent and 79 per cent, respectively.

activity potential present in the given tissue. Table II summarizes the mean per cent unit tissue homogenate enzyme activities compared to liver (assigning a value of 100 per cent to the liver) for each tissue studied in experiment I and experiment II.

The *succinic dehydrogenase* tissue homogenate activities of experiment I are given in Fig. 3. The results obtained categorize the tissues into 3 groups. Group 1 shows submaxillary gland, liver, and brain cortex to possess the highest unit enzyme activities, the brain cortex being somewhat lower than submaxillary gland and liver. Group 2 contains tooth pulp and Group 3 contains the gingiva, each possessing, respectively, approximately ½ and ¼ the unit activity of the submaxillary gland.

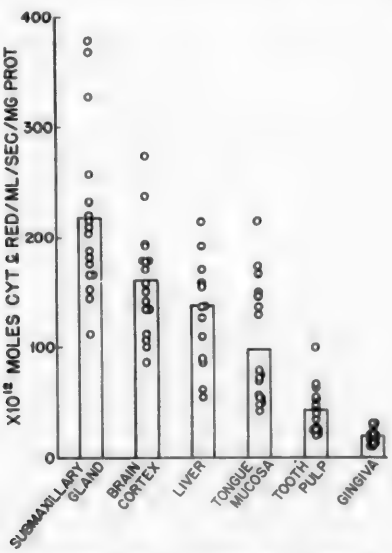


Fig. 4.—Succinic dehydrogenase unit activities of normal rabbit tissue homogenates, experiment II. The following amounts of tissue homogenate were employed: 0.05 ml. of liver, brain cortex, submaxillary gland and tongue mucosa, and 0.10 ml. of gingiva and tooth pulp. In these analyses, 0.58 mg. of pure cytochrome *c* was added to each cuvette. Otherwise, the conditions given for analysis in Fig. 3 remained the same.

The succinic dehydrogenase data for experiment II are summarized in Fig. 4. The data obtained here enable one to class the tissues in the following manner: submaxillary gland, brain cortex and liver, fall into Group 1 containing tissues of the highest activities. It should be noted, however, that the mean unit tissue enzyme activities for the 3 tissues are significantly different at the 5 per cent level. The tongue mucosa makes up Group 2, the tooth pulp Group 3, and the gingiva, Group 4. They contain approximately ½, ⅕, and $\frac{1}{12}$ the activity of submaxillary gland, respectively (Table II).

Fig. 5 presents the unit activity values for the *DPN cytochrome c reductase* assays performed upon each tissue in experiment I. The tissues can be classed in three groups based upon the enzyme activity levels found to be present.

Brain cortex gave the highest unit activity and is placed in Group 1, submaxillary gland and liver tissue fall into Group 2 possessing approximately ⅔ the activity of the brain cortex. Tooth pulp and gingiva constitute Group 3 and possess approximately 1/10 the activity of the brain cortex.

The unit DPN cytochrome *c* reductase activities obtained in experiment II are given in Fig. 6. The brain cortex was not studied in this series. Based upon these data, the tissues can be classed into 4 groups. Liver and submaxillary gland gave the highest unit activities and constituted Group 1, the latter possessing approximately ¾ the mean activity of the liver. The tongue mucosa represented Group 2, tooth pulp Group 3, and gingiva Group 4, each possessing approximately ⅕, 1/10, and 1/25 the mean unit activity of the liver, respectively (Table II).

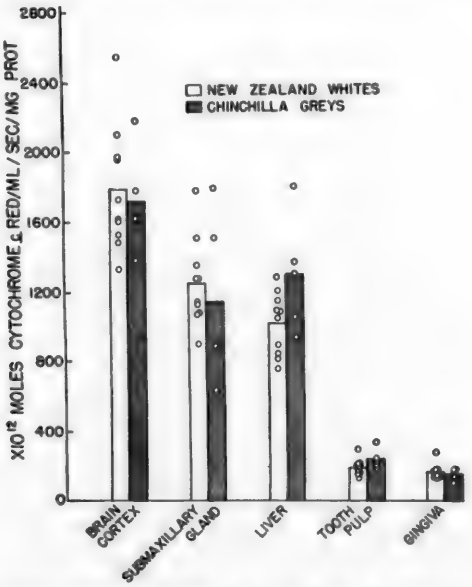


Fig. 5.—DPN cytochrome *c* reductase unit activities of normal rabbit tissue homogenates, experiment I. The reaction mixtures contained in the cuvettes were added in the following concentrations and sequence: 1.00 ml. of 0.15 M KH_2PO_4-Na_2HPO_4 buffer (pH 7.4 when diluted 1:3 times with H_2O); sufficient distilled H_2O to yield a total reaction volume of 3.0 ml.; 0.10 ml. of 3.0×10^{-2}M NaCN; 0.01 ml. of liver, brain cortex, and submaxillary gland, and 0.10 ml. of gingiva and tooth pulp tissue homogenates; 1.00 ml. ferricytochrome *c* (0.5 mg. of pure cytochrome *c* per 1.00 ml. of H_2O); 0.20 ml. of 0.82 M nicotinamide; 0.20 ml. of (red) DPN (1.0 mg. of [red] DPN* per 1.0 ml. of 0.05 M KH_2PO_4-Na_2PHO_4 buffer, pH 7.4). The calculated purity of the (red) DPN preparation showed it to be 70 per cent pure. The reduction of ferricytochrome *c* was followed at 550 mμ with a slit width of 0.01 mm.

The unit *cytochrome oxidase* activity data obtained in experiment I are given for each tissue in Fig. 7. Here the tissues comprised 3 groups. Group 1 included only brain cortex, the most active tissue. Group 2 included submaxillary gland and liver, possessing near comparable unit activities to that of the brain cortex. Group 3 included the tooth pulp, and gingival tissues, each possessing near equal unit activities but as a group decidedly lower than Group 2. The latter tissues contained approximately 1/10 the activity of brain cortex.

*(Red) DPN "90" obtained from the Sigma Chemical Co.

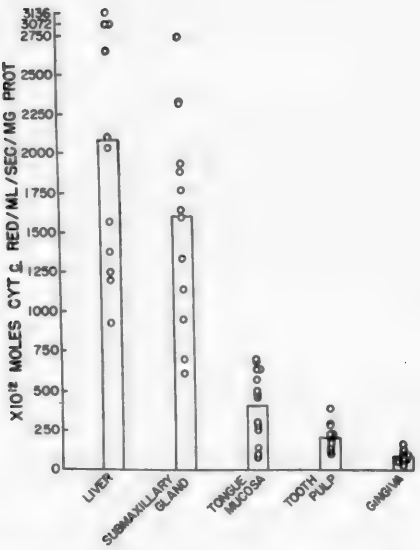


Fig. 6.—DPN cytochrome *c* reductase unit activities of normal rabbit tissue homogenates, experiment II. 0.025 ml. of tongue mucosa homogenate was employed. In addition, 0.58 mg. of pure cytochrome *c* was added to each cuvette. Otherwise, the conditions given for analysis in Fig. 5 remained the same.

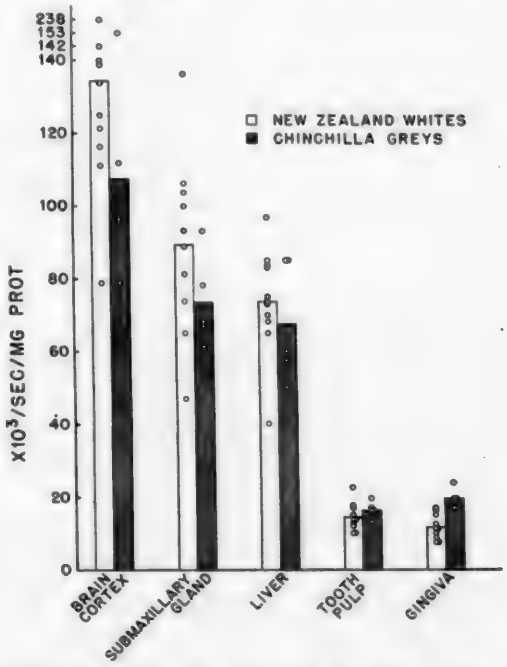


Fig. 7.—Cytochrome oxidase unit activities of normal rabbit tissue homogenates, experiment I. The reaction mixtures contained in the cuvettes were added in the following concentrations and sequence; 2.00 ml. of ferrocytochrome *c* (0.5 mg. pure cytochrome *c* per 1.0 ml. of H_2O plus 1.0 ml. of 0.15 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.4)*; sufficient water to yield a total reaction volume of 3.0 ml.; 0.01 ml. of liver, brain cortex and submaxillary gland and 0.10 ml. of gingiva and tooth pulp tissue homogenates. The oxidation of ferrocytochrome *c* was followed at 550 mμ with a slit width of 0.01 mm.

*The cytochrome *c* was reduced in solution by the addition of a pinch of sodium hydrosulfite ($Na_2S_2O_4$) followed by aeration for 5 minutes to oxidize the excess $Na_2S_2O_4$.

For experiment II the unit cytochrome oxidase activities are given in Fig. 8. Brain cortex was not studied in this series. The tissues may be categorized into 4 groups of decreasing activity, respectively: submaxillary gland yielded the highest unit activity and comprised Group 1; liver comprised Group 2; tongue mucosa, Group 3; and the tooth pulp and gingiva, Group 4. The latter tissues possessed approximately $\frac{2}{3}$, $\frac{1}{3}$, and $\frac{1}{15}$ the mean unit activity of the submaxillary gland (Table II).

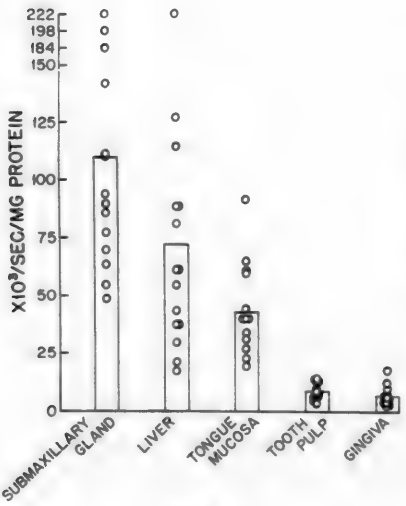


Fig. 8.

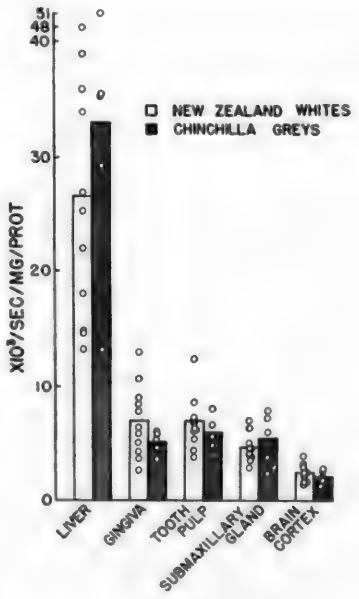


Fig. 9.

Fig. 8.—Cytochrome oxidase unit activities of normal rabbit tissue homogenates, experiment II. 0.025 ml. of tongue mucosa homogenate was employed. Each reaction cuvette contained 0.58 mg. of pure cytochrome *c*. Otherwise, the conditions given for analysis in Fig. 7 remained the same.

Fig. 9.—Catalase unit activities of normal rabbit tissue homogenates, experiment I. The reaction mixture contained in the cuvettes were added in the following concentrations and sequence: 1.00 ml. of H_2O_2 solution (0.15 ml. of 28 per cent superoxal in 25.0 ml. of 0.05 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.1); 1.90 ml. of 0.05 M KH_2PO_4-Na_2HPO_4 buffer, pH 7.1; and 0.10 ml. of each tissue homogenate. The decomposition of H_2O_2* was followed at 240 mμ with a slit width of 0.3 mm.

The individual and mean unit *catalase* activities of experiment I are presented in Fig. 9. The tissues may be separated into 3 groups based upon the activity levels found. Liver possessed the highest unit catalase activity and is classed in Group 1. Group 2 included gingiva, tooth pulp, and submaxillary gland all of near equal activity. Each of these tissues contain approximately $\frac{1}{5}$ the activity of the liver. Brain cortex possessed $\frac{1}{10}$ the activity of liver, the smallest activity detected, and made up Group 3 (Table II).

*The quartz cuvettes used in the catalase assays were cleaned by immersion in chromic acid, rinsed thoroughly with distilled H_2O, rinsed several times with acetone and air dried to minimize collection of oxygen bubbles upon the cuvette walls.

DISCUSSION

The identification of succinic dehydrogenase activity in these tissues implicates a functional tricarboxylic acid cycle (Krebs cycle) in their metabolism of carbohydrates. Since succinic dehydrogenase is localized in the mitochondria of the cell in association with cytochrome oxidase, the demonstration of succinic dehydrogenase activity automatically implicated the presence of a functional cytochrome respiratory system in each of the oral tissues studied.

The direct observation of succinic dehydrogenase activity in the tooth pulp corroborates the report of Pincus.[3] Its direct demonstration in submaxillary gland agrees with the observations of Broch, Druckrey, and Loch,[1] Jungueria,[2] and Person and Burnett.[4] No references were found which describe the identification of succinic dehydrogenase activity in the gingiva and tongue mucosa.

The presence of DPN cytochrome *c* reductase in submaxillary gland, gingiva, tooth pulp, and tongue mucosa demonstrates that they possess the capacity to mediate the transfer of electons between (red) DPN and oxidized cytochrome *c*. This observation further supported the probability that a functional cytochrome respiratory system should exist in these tissues.

The detection of cytochrome oxidase activity in tissues provides evidence that the cell is capable of utilizing oxygen under physiologic conditions. Mechanisms other than cytochrome oxidase (e.g., flavoprotein-type oxidases) are also present in the cell and may account for some oxygen utilization. However, at the oxygen tensions within the cell it is unlikely that they are of too great importance physiologically (Theorell,[15] Eichel, and Waimio[16]). The spectrophotometric assay of cytochrome oxidase further provides direct evidence for a tissue's capacity to oxidize reduced cytochrome *c* in the presence of oxygen.

Confirmation of a functional cytochrome oxidase system for liver and brain cortex has been obtained. Evidence for the presence of this enzyme in submaxillary gland, tooth pulp, tongue mucosa, and gingiva has been established. Person and Burnett[4] have also recently reported the detection of cytochrome oxidase in submaxillary gland. The previous report of Glickman, Turesky, and Hill,[17] that normal human gingiva possessed the capacity to consume oxygen, was the only data available which suggested the presence of oxygen activating mechanisms in this tissue.

Catalase probably serves a peroxidatic function in mammalian tissues in vivo[18-20] by decomposing the H_2O_2 produced as an end product of flavoprotein mediated dehydrogenations between substrate and molecular oxygen (primary oxidation), with the ultimate formation of active oxygen. Apparently the latter can directly oxidize suitable substrates (secondary oxidation) without additional enzyme intervention.

The catalase activities as determined in the tissue homogenates by the method of Beers and Sizer[12] involve the decomposition of H_2O_2 with the formation of molecular oxygen. Unfortunately this assay gave the most variable results of the enzyme systems reported here. Two factors probably caused this. First, interference with the H_2O_2 ultraviolet absorption spectrum by the tissue

homogenate protein probably occurred. Second, unknown side reactions may have also taken place. This difficulty, undoubtedly, was not encountered by Beers and Sizer since they had adapted the method for the study of pure solutions of the catalase enzyme. However, the very high liver unit catalase activities observed in this study are consistent with the observations of others. The results obtained also demonstrate that catalase activity is present in the tooth pulp, gingiva, and submaxillary gland.

Good reproducibility was obtained for succinic dehydrogenase, DPN cytochrome *c* reductase, cytochrome oxidase, catalase and total protein mean levels when statistical comparisons were made between the New Zealand white and chinchilla gray rabbits of experiment I. Only gingiva cytochrome oxidase activity (Fig. 7) and total protein (Fig. 1) were higher in the chinchilla gray rabbits. Thus, out of 25 possible comparisons between the 2 rabbit strains, only 2 appeared to be different at the .05 level. These differences may well be due to chance since 1 or 2 of 25 comparisons may test significantly different when in fact they are not. Thus, the conclusion may be reached that no difference existed between the 2 strains of rabbits studied in experiment I.

In general, the results obtained in experiment II confirmed the group relationships established in experiment I (Tables I and II). The total protein relationships established in Table I yield the impression that the data collected in experiments I and II are identical. It should be noted, however, that the total protein values for experiment II, Fig. 2 are uniformly higher than those for experiment I, Fig. 1. This is probably explained by the fact that a modification in the technique of weighing the tissues was incorporated into experiment II. In experiment I, the paraffinized paper was weighed prior to the weighing of the combined tissue plus the paper. Consequently, residual moisture and/or particles of tissue may have been retained on the weighing paper after placement of the tissue mass into the homogenizer. In experiment II, after taking the combined weight of the tissue and the paper, the paper alone was weighed. Thus, a proper correction for any residual moisture and/or tissue that remained on the paper was made for the tissues taken in the second experiment. It is believed that the total protein values reported in Fig. 2 more closely approximate the true protein content of the tissues.

Those tissues which yielded high or low enzyme activities in experiment I clearly show high or low enzyme activities in experiment II, although some variation did occur, Table II. The high activity levels for the submaxillary gland succinic dehydrogenase, DPN cytochrome *c* reductase and cytochrome oxidase enzymes compared favorably to the same enzyme activities in brain cortex and liver. This relationship was expected since glandular tissues should require a great energy supply. In addition, the major cell type which makes up the submaxillary gland is one which should be associated with high metabolic activity. The low catalase activity found in the submaxillary gland as compared to liver was not expected. Only the brain cortex possessed lower catalase activity than did the submaxillary gland. Tooth pulp and gingiva on the other hand possessed slightly greater catalase activity than did the submaxillary gland

in the New Zealand white rabbits. Yet, in terms of succinic dehydrogenase, DPN cytochrome *c* reductase, and cytochrome oxidase enzyme activities the tooth pulp and gingiva were definitely of a lower order of magnitude than the submaxillary gland. The same relationship exists when tooth pulp and gingiva are compared to the liver and brain cortex.

The catalase activities in the tooth pulp and gingiva were found to be the same. The gingiva cytochrome oxidase activity of the chinchilla gray rabbits was found to be higher than that of the tooth pulp. This finding may be due to chance. In all other comparisons, the tooth pulp contained greater enzyme activity than did the gingiva. Since much of the gingival tissue mass is dense fibrous connective tissue, the latter relationship is not surprising. However, if it were possible to separate the fibrous tissue from the epithelial tissue layers of the gingiva, higher enzyme activities would undoubtedly be detected than those activities identified here.

It should be noted that a definite nonhomogenizable residue was routinely detected following the homogenization of each gingiva and tongue mucosa sample. It is possible that this residue consisted mainly of (a) collagen, (b) keratinized material, or (c) a combination of both. If the residue had been disintegrated and evenly distributed during homogenization, it is probable that the total protein values obtained would have been higher and conversely the unit enzyme activities would have been lowered. It is assumed, however, that a considerable portion of the dermis collagen and keratinized epithelial tissue was disrupted and homogeneously distributed throughout the tissue suspension.

The enzyme activities detected in the tongue mucosa are considered relatively high and were greater than those found in the tooth pulp and gingiva. Since the muscle tissue was as carefully scraped away from the tongue mocosa as possible, it is reasonable to attribute to the mucosa the enzyme activities observed. However, as with the gingiva, were it possible to separate completely the dense fibrous connective tissue from the epithelial layers, the tongue mucosa should yield high enzyme activity levels. The high metabolic activity of the tongue mucosa may be intimately related to the common occurrence of clinical signs and symptoms of nutritional deficiencies in this tissue.

SUMMARY

1. Total protein, succinic dehydrogenase, DPN cytochrome *c* reductase, cytochrome oxidase, and catalase have been determined in submaxillary gland, tooth pulp, gingiva, tongue mucosa, liver, and brain cortex tissue homogenates of normal female rabbits.

2. Total protein was quantitated in terms of gamma of protein per 0.1 ml. of a 1:50 dilution of homogenate. Enzyme concentrations were expressed as unit activities, the enzyme velocity constant per milligram of protein.

3. Comparisons were made between each tissue to establish the relative concentrations for each component analyzed, assigning a value of 100 per cent to the liver.

4. The liver and submaxillary gland possessed equally high levels of total protein. Tongue mucosa, brain cortex, gingiva and tooth pulp, respectively, gave lower total protein values; the brain cortex and gingiva being approximately equal.

5. The liver, submaxillary gland, and brain cortex possessed high succinic dehydrogenase, DPN cytochrome *c* reductase, and cytochrome oxidase unit activities. The tongue mucosa yielded intermediate activities for these enzymes; whereas, relatively low activities were uniformly detected in the tooth pulp and gingiva.

6. For catalase, the liver yielded the highest unit enzyme activity. Gingiva, tooth pulp, and submaxillary gland exhibited considerably lower unit activity. The brain cortex possessed the smallest activity.

REFERENCES

1. Broch, N., Druckrey, H., and Loch, W.: The Effect of Different Substrates on Tissue Metabolism, *Biochem. Ztschr.* **313**: 300-316, 1943.
2. Jungueria, L. C. U.: Cytological, Cytochemical, and Biochemical Observations on Secreting and Resting Salivary Glands, *Exp. Cell Res.* **2**: 327-338, 1951.
3. Pincus, P.: Enzymes in Dentine and Pulp, *Brit. D. J.* **90**: 201-205, 1951.
4. Person, P., and Burnett, G. W.: Cytochrome Oxidase and Succinoxidase Activity in Oral Tissues, *J. D. Res.* **33**: 680, 1954.
5. Eichel, B., and Swanson, A. A.: Succinic Dehydrogenase, DPH Cytochrome *c* Reductase, Cytochrome Oxidase, Catalase and Total Protein of Retina and Choroid, USAF School of Aviation Medicine, Report No. 56-71, 1956. (Lens Tissue Data—In press.)
6. Eichel, B.: Oxidative Enzymes in Tooth Pulp, Gingiva and Submaxillary Gland, *J. D. Res.* **33**: 656, 1954.
7. Eichel, B., and Swanson, A. A.: Intracellular Oxidative Enzymes in Oral Tissues. II. Succinic Dehydrogenase, DPN Cytochrome *c* Reductase, Cytochrome Oxidase and Total Protein in Tongue Mucosa, *J. D. Res.* **34**: 682, 1955.
8. Kabat, E. A., and Mayer, M. M.: Experimental Immunochemistry, Springfield, Ill., 1948, Charles C Thomas, Publisher, chapt. 22.
9. Cooperstein, S. J., Lazarow, A., and Kurfess, N. J.: A Microspectrophotometric Method for the Determination of Succinic Dehydrogenase, *J. Biol. Chem.* **186**: 129-139, 1950.
10. Potter, V. R., and Albaum, H. G.: Studies on the Mechanism of Hydrogen Transport in Animal Tissues. VII. Inhibition by Ribonuclease, *J. Gen. Physiol.* **26**: 443-455, 1943.
11. Wainio, W. W., Person, P., Eichel, B., and Cooperstein, S. J.: The Reaction Between Cytochrome Oxidase and Ferrocytochrome *c*, *J. Biol. Chem.* **192**: 349-359, 1951.
12. Beers, R. F., and Sizer, I. W.: A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase, *J. Biol. Chem.* **195**: 133-139, 1952.
13. Tint, H., and Reiss, W.: Studies on the Purity and Specificity of Cytochrome *c*. II. *J. Biol. Chem.* **182**: 397-403, 1950.
14. Umbreit, W. W., Burris, R. H., and Stauffer, J. F.: Manometric Techniques and Tissue Metabolism, Minneapolis, 1949, Burgess Publishing Co., chapt. 16, p. 214.
15. Theorell, H.: Keilin's Cytochrome *c* and the Respiratory Mechanism of Warburg and Christian, *Nature* **138**: 687, 1936.
16. Eichel, B., and Wainio, W. W.: D-Glucose Dehydrogenase and Its Carrier Systems, *J. Biol. Chem.* **175**: 155-168, 1948.
17. Glickman, I., Turesky, S., and Hill, R.: Determination of Oxygen Consumption in Normal and Inflamed Human Gingiva Using the Warburg Manometric Techniques, *J. D. Res.* **28**: 83-94, 1949.
18. Keilin, D., and Hartree, E. F.: Coupled Oxidation of Alcohol, *Proc. Roy. Soc. London.* **B119**: 141, 1936.
19. *Idem:* Properties of Catalase, Catalysts of Coupled Oxidation of Alcohols, *Biochem. J.* **39**: 293-301, 1945.
20. Chance, B.: The Reaction of Catalase in the Presence of the Notatin System, *Biochem. J.* **46**: 387-402, 1950.

THE MINERALIZATION OF RAT ENAMEL IN THE LIGHT OF CA^{45} AUTORADIOGRAPHY AND MICROINCINERATION

LEONARD F. BÉLANGER

Department of Histology and Embryology, School of Medicine, University of Ottawa, Ottawa, Canada

THE mechanism of mineralization of enamel is still not clearly understood. It is generally agreed that it takes place over a prolonged period and the term "maturation" is utilized to describe the changes which occur after the secretion of the primary substance. According to Leicester[1] who summarized the opinions of the majority of authors, once mineralization of the matrix begins, it continues as a steady, gradual process. There are, however, investigators[2, 3] who described a "secondary calcification" as a separate event taking place in the portion of enamel which has achieved full width. The present series of observations, combined with previously reported data,[4-9] casts some light on this aspect of the problem as well as on that of the matrix-minerals relationships and on the behavior of the enamel organ.

MATERIALS AND TECHNICS

Three hamsters (Cricetus auratus) of 4 days of age (average weight, 4 Gm.), 5 white rats (Sprague-Dawley) of 6 days (av. wt., 16 Gm.) and 2 rats of 10 days (av. wt., 27 Gm.) were injected subcutaneously with a single dose of 3 μc per gram of Ca^{45} as calcium chloride in HCl with a high specific activity. These suckling animals were returned to their mothers and sacrificed at intervals of 2 hours to 6 days afterward. The dissected tissues were fixed in formaldehyde-ethanol for 48 hours,[11] dehydrated in graded ethanol, and embedded in low viscosity nitrocellulose for sectioning. Integrated autoradiographs of 12 μ sections were obtained by the coating-inversion technic.[10-12] Other sections were incinerated by the method of Policard-Scott[13] and examined by dark field microscopy.

OBSERVATIONS

The *autoradiographic record* of entry and transit of radiocalcium in dentin was essentially similar to that previously reported in incisors of normal pigs[14] and also comparable to the observations on young rats (Kumamoto and Leblond[15]).

The entry of Ca^{45} into the enamel of the 4-day-old hamsters and 6-day-old rats was generally in the form of a diffuse band of lower intensity than that of dentin, extending more or less along the whole length of the substance and located at some distance from the surface. This wide band showed also a gradient of intensity toward the dentinoenamel junction.

Received for publication June 6, 1956.

In the 2 posterior cusps of the first molar in the rat of 6 days of age, there was a more intense and wider reaction at the apex, facing the small ameloblasts. This zone was also recognized in animals sacrificed 2, 4, and 6 days after the introduction of tracer.

With time (up to 6 days), the localization of the radioelement seemed to change very little, except that the new increment of enamel had made the layer thicker, giving the impression that the labeled area was more deeply located.

The observations of early entry (2 hours) in the molars of the 10-day-old rat were more varied. At that age, there are pronounced degrees of "maturation" between the first and the second molar, even between the cusps of the same tooth. In the young tissue of the second molar (Fig. 1, *M2, a*), the picture previously described could be recognized in the new, distal portion of the tissue. Toward the apex, the autoradiographic record decreases and eventually disappears to reappear at the very top, much stronger. In the first cusp of the first molar (Fig. 1, *M1, a*), these 2 events can also be recognized.

Fig. 1.—Integrated autoradiograph (unstained) of the first and second molar, upper jaw, from a 10-day-old rat, sacrificed 2 hours after an injection of $Ca^{45}Cl_2$. (×29.)

Here though, the apical zone is wider than in *M2, a*. In the second and third cusps (Fig. 1, *M1, b* and *c*), the apical localization is the only one visible. There is a gradient in this secondary image from *a* to *b* to *c:* the autoradiographic record is progressively more intense and covers a wider area of the enamel. In *M1, b* and *c* (Fig. 1), it extends distally in the form of a thin band located at a regular distance from the surface. This extreme distal extension of the secondary image corresponds to the presence of a bridge of short ameloblasts, (Fig. 1, arrows) raised in a semicircle above the surface of the enamel and previously described as "gap."[9] Apart from these areas of greater intensity, the enamel has shown a general low intensity record. Dentin and bone have also produced a general low intensity record except at the accretion surfaces where the image is considerably darker (Fig. 1).

When the sections were preincubated for 3 hours in a formic-citrate bath at pH 4.9, counts with the Geiger-Mueller apparatus have been reduced to background; also none of the aforementioned autoradiographic localizations have been obtained.

Microincineration.—The ash picture (spodogram) revealed practically no minerals in the odontoblasts. Their lateral limits, however, were well marked by parallel bands of white ash apparently related to the intercellular cement and previously observed in the teeth of pigs.[14] By opposition, the ameloblasts exhibited a cellular content of fine ash, apparently more abundant in the large cells distal to the gap. In these, the ash seemed evenly distributed, while in the smaller ameloblasts, above the gap, the ash appeared only in the basal portion of the cells.

In the young animals, the superficial portion of the enamel of the molar teeth (probably pre-enamel[16, 17]) appeared devoid of minerals while the inner portion showed a progressive concentration toward the dentinoenamel junction.

In the 10-day-old rats, a similar pattern was observed in the youngest distal portion of the tissue (Figs. 2 and 3). At the level of the gap, a high concentration of minerals appeared in the form of a band occupying presumably the zone of young enamel and appearing interposed between two parallel bands of lower mineral concentration (Figs. 2 and 3). The initial distal band of minerals enlarged progressively and eventually joined with this secondary deposit. Past the area of the gap toward the apex, the mineral deposit occupied progressively the whole surface of enamel and appeared to be increasing in concentration (Fig. 3) to eventually match, in density, the uniformly highly mineralized dentin.

The spodogram of the incisor tooth of the upper jaw appeared different. The ash deposit, originally a thin line at the dentinoenamel junction, increased steadily in thickness and in concentration until it occupied the whole width of the enamel.

DISCUSSION

The interpretation of autoradiographs is subject to recognition of atomic interexchange and actual accretion by biosynthesis. Evidence has been obtained previously[6] to the effect that, when mineralized sections of bones and teeth are placed in a solution of $Ca^{45}Cl_2$, radioactive uptake is greater in areas of lesser mineralization. In the present material, the radioactive component is entirely labile in weak acid and most likely mineral in greatest part. The autoradiographic pattern, obtained from the teeth of young animals, indicates a possible correlation between total mineralization and intensity of the autoradiograph in the enamel but not in the dentin. In that tissue as well as in bone, numerous reports[14, 15, 18, 19] have shown the accretion surfaces as the most active sites, indicating that most of the radioactive substance present is being biosynthetized locally. This view has been confirmed by experiments in which the deposition of organic large molecules actually required a complex biochemical mechanism.[7-9] Consequently, it may be assumed that the enamel picture represents largely accretion.

In the early stage of enamel formation, there appears to be mineral deposition taking place sometime after the formation of organic matrix. On the other hand, in the molars of the 10-day-old rat, particularly in the first

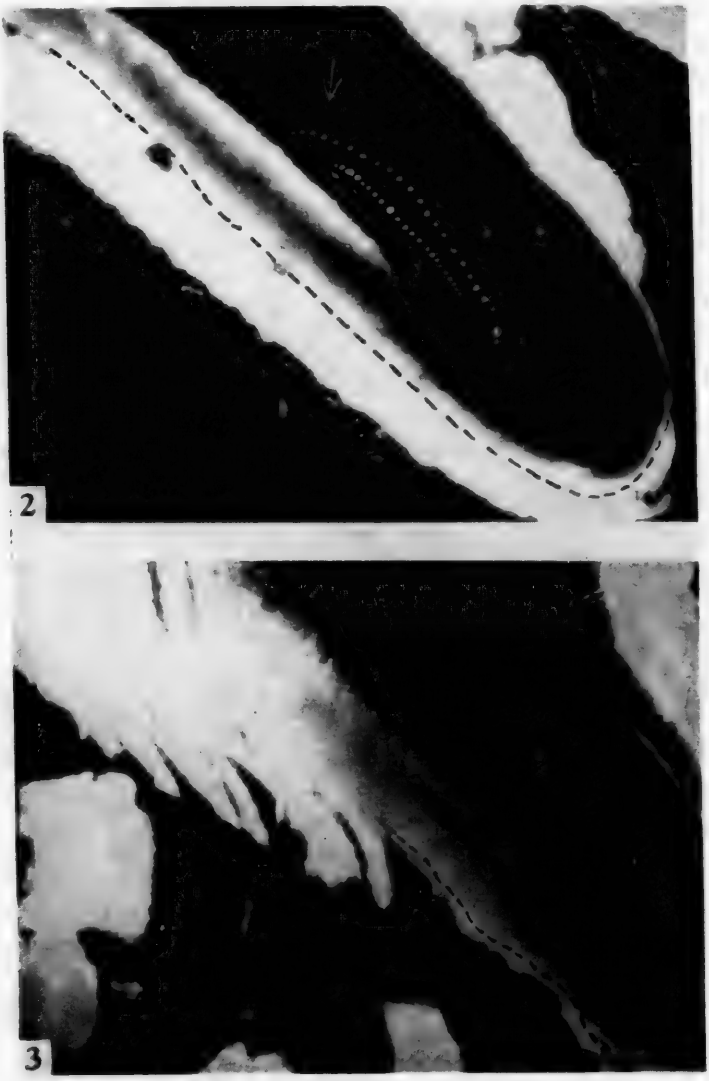


Fig. 2.—Spodogram of part of a section of cusp *M1*, *b* (Fig. 1). The limits of the gap and the dentinoenamel junction have been outlined. (×99.)
Fig. 3.—Spodogram of part of a section of cusp *M1*, *c*, treated as above. (×99.)

molar, it seems that a secondary and more intense process of mineralization begins later at the level of young enamel. This in turn is followed by deposition of minerals throughout the whole tissue.

The spodogram of different parts of the molar tooth enhances the autoradiographic demonstration of a triphasic operation.

At the dentinoenamel junction of the youngest most distal portion of the tissue, a thin band of ash is visible. This band increases slowly in width and density. At a distance from the original formative site, the enamel reveals the presence of another deposit of minerals, linear in appearance (Figs. 2 and 3) and located between two zones of lower mineral content. Farther up toward the apex of the cusp, this band is joined by increasing minerals from underneath and disappears as a visible entity as the mineral concentration increases progressively toward the apex and toward the surface of the tooth.

In previous work, evidence of this secondary phase of mineralization has been obtained without being fully recognized. In a paper dealing with autoradiographic mapping of P^{32} uptake by the teeth,[5] we had observed in the enamel of the first molar of a 6-day-old rat "an area of higher intensity at the junction of approximately the outer one third with the inner two thirds," persisting 2 days after the introduction of tracer. A silver nitrate reaction on the first molar of a 4-day-old hamster produced "two distinct zones, one at the dentinoenamel junction, the other one near the surface of the enamel."[5] Furthermore, the experiments with Ca^{45} in vitro intake have demonstrated, inside the enamel of human molars, zones in which the mineralization was apparently lesser than that of the surface.[6] All these observations on the molar teeth are in favor of the opinion expressed by Mellanby,[2] and by Diamond and Weinmann[3] to the effect that mineralization of enamel is the result of a double operation. The fact that the second phase of mineralization begins opposite the semicircular gap (Figs. 1, 2, and 3) appears significant. This feature of the enamel organ has been originally described by Marsland[20] as marking the end of matrix formation and the beginning of calcification. Such areas of raised ameloblasts have been observed by us in different portions of the enamel organ:

1. Immediately before the beginning of organic secretion.[8]

2. At the end of secretion of the organic substance labeled with $S^{35}O_4$ (sulfomucoprotein); this site corresponds also to the cessation of incorporation of S^{35} methionine[9] (enamel protein), to the cessation of production of alkaline phosphatase by the enamel organ,[8] and, finally, this second and most frequently observed location corresponds to the onset of the presently described secondary phase of mineralization (Figs. 1, 2, and 3).

3. A gap has also been seen occasionally at the end of the secondary phase of mineralization, at a point where the whole substance of enamel appears to be recipient to mineral deposition.

4. Finally, more or less large areas of detached epithelium may appear anywhere along the enamel organ as the result of histologic technic. These last mentioned artefacts can be distinguished from the functional sites by the appearance of the ameloblasts.[8]

The existence of these sites where the ameloblasts change in appearance, where certain functions begin or cease, indicate clearly that the enamel organ is a multiphasic structure which proceeds by steps toward the formation of enamel. In the molars of 10-day-old rats, 3 episodes can be detected; (1) the secretion of a substance (pre-enamel) which after a time is receptive to minerals (young enamel), (2) the cessation of organic increment and the beginning of secondary mineralization farther away in young enamel, and (3) a phase of progressive receptivity to mineral deposition throughout the enamel substance (transitional enamel and mature enamel).

The factors responsible for the last phase are not revealed by the present experiments. There is new evidence toward the fact that mineralization has something to do with the quality of the organic matrix. It has been established previously that the main constituents of this matrix are the filamentous protein keratin[1] and a mucoprotein[21] which appears sulfated.[8] This last substance has a rapid turnover as compared to the former.[8, 9] Since the mucoprotein mostly occupies the superficial portion of the enamel, it seems likely that its presence is unfavorable to mineral deposition and that its gradual disappearance renders the enamel receptive to mineralization. A similar situtation prevails in cartilage at the epiphyseal plate.[7, 22, 23]

It appears evident from the present studies (Fig. 1) that, before the tooth is erupted, the radioactive calcium is deposited via the enamel organ. The relatively high concentration of white ash observed in the spodograms of the large ameloblasts and previously reported by Hampp[24, 25] does not necessarily imply a relationship with mineralization. Similar high ash contents have been observed under comparable conditions in other highly active epithelial derivatives such as the hair follicle and the retina.[26] However, the fact that practically no Ca^{45} incorporation is evident at the level of the gap (Fig. 1, *M1, a, M2, a*) seems to indicate variations in the ameloblasts during mineral deposition.

Finally, the constantly growing incisor has shown, in previous work,[8] the presence of the first and second gap associated with the formation of organic enamel but the present study has failed to demonstrate, by autoradiography or spodography, the second of the 3 phases of mineralization described in the molars. Mineralization in the incisor seems to proceed upward from the dentinoenamel junction by a progressive, regular process. Also, the thickness of the enamel increases more slowly.

SUMMARY

The mineralization of enamel appears different in the incisors and molars of young rats when viewed in the light of Ca^{45} autoradiography and also by microincineration.

In the incisor, the process is regular and progressive.

In the molar, the initial slow process is followed by massive mineralization of young enamel when the organic matrix stops being secreted. This is followed by progressive general mineralization of the completed organic structure.

The various phases in the life of the enamel organ are often morphologically indicated by the presence of successive semicircular gaps.

Before eruption, the enamel receives its minerals via the enamel organ.

The author is indebted to Mrs. Cécile Bélanger for skilled technic and to the Associate Committee for Dental Research of the National Research Council of Canada for financial assistance. The radiocalcium was purchased from Atomic Energy of Canada, Ltd.

REFERENCES

1. Leicester, H. M.: Biochemistry of the Teeth, St. Louis, 1949, The C. V. Mosby Co.
2. Mellanby, M.: Diet and the Teeth: An Experimental Study, Part 1. Dental Structure in Dogs, London M. Research Council, Special Report, Series No. 140, 1929.
3. Diamond, M., and Weinmann, J. P.: The Enamel of Human Teeth, New York, 1940, Columbia University Press.
4. Bélanger, L. F., and Leblond, C. P.: Mineralization of the Growing Tooth as Shown by Radio-Phosphorus Autographs, *Proc. Soc. Exper. Biol. & Med.* **73**: 390, 1950.
5. Bélanger, L. F.: Autoradiographic and Histochemical Observations on the Mineralization of Teeth in Rats and Hamsters of Various Ages, *Anat. Rec.* **114**: 529, 1952.
6. *Idem:* Autoradiographic Visualization of In Vitro Exchange in Teeth, Bones, and Other Tissues, Under Various Conditions, *J. D. Res.* **32**: 168, 1953.
7. *Idem:* Autoradiographic Visualization of the Entry and Transit of S^{35} in Cartilage, Bone, and Dentine of Young Rats and the Effect of Hyaluronidase In Vitro, *Canad. J. Biochem. & Physiol.* **32**: 161, 1954.
8. *Idem:* Autoradiographic Detection of Radiosulfate Incorporation by the Growing Enamel of Rats and Hamsters, *J. D. Res.* **34**: 20, 1955.
9. *Idem:* Autoradiographic Visualization of the Entry and Transit of S^{35} Methionine and Cystine in the Soft and Hard Tissues of the Growing Rat, *Anat. Rec.* **124**: 555, 1956.
10. Bélanger, L. F., and Leblond, C. P.: A Method for Locating Radioactive Elements in Tissues by Covering Histological Sections With a Photographic Emulsion, *Endocrinology* **39**: 8, 1946.
11. Bélanger, L. F.: A Method for Routine Detection of Radiophosphates and Other Radioactive Compounds in Tissues. The Inverted Autograph, *Anat. Rec.* **107**: 149, 1950.
12. *Idem:* Improvements to the Melted Emulsion Technique of Autoradiography, *Nature* **170**: 625, 1952.
13. McClung-Jones, R.: McClung's Handbook of Microscopical Technique, ed. 3, New York, 1950, Paul B. Hoeber, Inc.
14. Bélanger, L. F., Lotz, W. E., Visek, W. J., and Comar, C. L.: Autoradiographic Visualization With Ca^{45} of Normal Growth of the Incisor of Pigs and the Effect of Fluorine Feeding, *Anat. Rec.* **119**: 53, 1954.
15. Kumamoto, Y., and Leblond, C. P.: Radioautographic Study of Mineralization of Growing Teeth With Labeled Calcium, *J. D. Res.* **35**: 147, 1956.
16. Chase, S. W.: The Nature of the Enamel Matrix at Different Ages, *J. A. D. A.* **22**: 1343, 1935.
17. Leblond, C. P., Bélanger, L. F., and Greulich, R. C.: Formation of Bones and Teeth as Visualized by Radioautography, *Ann. New York Acad. Sc.* **60**: 629, 1955.
18. Leblond, C. P., Wilkinson, G. W., Bélanger, L. F., and Robichon, J.: Radio-autographic Visualization of Bone Formation in the Rat, *Am. J. Anat.* **86**: 289, 1950.
19. Comar, C. L., Lotz, W. E., and Boyd, G. A.: Autoradiographic Studies of Calcium, Phosphorus and Strontium Distribution in the Bones of the Growing Pig, *Am. J. Anat.* **90**: 113, 1952.
20. Marsland, E. A.: A Histological Investigation of Amelogenesis in Rats. I. Matrix Formation, *Brit. D. J.* **91**: 251, 1951.
21. Pincus, P.: Relation of Enamel Protein to Dental Caries, *Nature* **161**: 1014, 1948.
22. Sylven, B.: Cartilage and Chondroitin Sulfate, *J. Bone & Joint. Surg.* **29**: 745, 1947.
23. Amprino, R.: Autoradiographic Research on the S^{35} Sulfate Metabolism in Cartilage and Bone Differentiation and Growth, *Acta. Anat.* **24**: 121, 1955.
24. Hampp, E. G.: Mineral Distribution in Developing Teeth, *Anat. Rec.* **77**: 273, 1940.
25. *Idem:* Micro-incineration Studies of an Enamel-Containing Ameloblastoma, *J. A. D. A.* **29**: 1819, 1942.
26. Bélanger, L. F.: The Entry of Ca^{45} Into the Skin and Other Soft Tissues: An Autoradiographic and Spodographic Study, *J. Histochem. & Cytochem.* **5**: No. 1, 1957.

TISSUE RESPONSE TO AIRBRASIVE POWDER, TO PULVERIZED ENAMEL AND DENTIN

DONALD A. KERR, D.D.S., M.S., SIGURD RAMFJORD, L.D.S., PH.D., AND GRETA GRAPE-RAMFJORD, L.D.S., M.S.

University of Michigan School of Dentistry, Department of Oral Pathology and Periodontia, Ann Arbor, Mich.

INTRODUCTION

WHEN the airbrasive technic for cavity preparation is used, there is always a hazard that some of the powder, tooth substance, and old filling material will be inhaled or introduced into the adjacent gingival tissues. It seemed desirable, therefore, to investigate tissue reactions to these substances.

According to specifications from the manufacturer of the airbrasive powder,* it contains Al_2O_3—94.56 per cent, SiO_2—2.23 per cent, TiO_2—2.75 per cent, FeO—0.41 per cent, CaO—trace. The particle size is specified by the company to be between 10 to 50 microns (average, 40 microns). A cursory study of the powder smeared on a glass slide and placed under a microscope revealed that there were numerous particles present with particle size less than 10 microns, but no conclusive study was undertaken on particle size. One component of the formula, silica, is known to produce definite toxic effects resulting in a granulomatous reaction. The effect of aluminum oxide is controversial. It is claimed to be inert[1] by some investigators and toxic by others.[2-4]

That implanted teeth are gradually resorbed is well established, but no report could be found on the fate of finely pulverized enamel and dentin implanted into living tissues. In reaction to dust, particle size is a very important factor with increasing tendency for granulomatous reaction with smaller particle size.[1]

METHOD

It has been established by McCord and others[5, 6] that Miller and Sayers'[7] method of intraperitoneal injection of sterile dust in saline suspension is the most satisfactory way to test general tissue reaction to a particulate foreign material. These authors[8, 9] considered 60 to 90 days to be a sufficient time interval for evaluation of tissue reaction to a given dust.

For this study, 59 adult guinea pigs were divided into 4 groups and intraperitoneal injections of the various test materials were administered. Each pig in Group I (18 guinea pigs) received 0.2 gram of new airbrasive powder in saline suspension. In Group II (14 guinea pigs) each received 0.2 gram of used airbrasive powder. In Group III (13 guinea pigs) each received, in saline

This report is from part of an investigation which was carried out under contract DA-49-007-MD-138 with the Medical Research and Development Board, Office of the Surgeon General of the United States Army.

Received for publication Aug. 25, 1956.

*S. S. White Dental Manufacturing Company, 211 South Twelfth Street, Philadelphia 5, Pa.

suspension, 0.2 gram of pure powdered enamel that had been passed through a 350-mesh sieve (allowing passage of particle sizes up to 35 to 38 microns). Each pig in Group IV (14 guinea pigs) received 0.2 gram of dentin powder which also had been passed through a 350-mesh sieve.

The animals were killed after 90 days and necropsy performed. Specimens from the lower abdominal wall, the omentum, the liver, and the spleen were removed for microscopic study.

Using an ordinary airbrasive unit, a stream of new airbrasive powder was directed at the gingival tissues of 5 adult rabbits at 2 places on each animal. The rabbits were killed after 50 to 365 days, and sections for microscopic study were prepared from 2 of the animals. The specimens included the jaws, teeth, and gingiva at the sites of the deposited airbrasive powder.

The sections were stained routinely with hematoxylin and eosin, and some of the sections from each group were stained with Azan connective tissue stains.

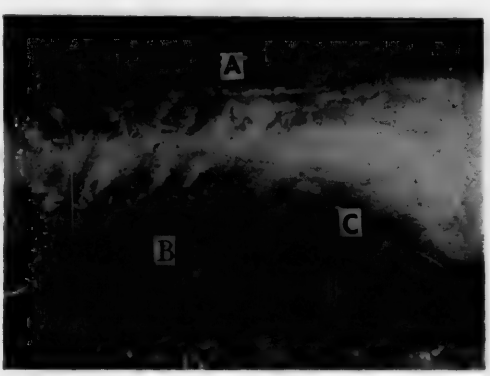


Fig. 1.—Plaques (*A, B, C*) of airbrasive powder adherent to the low abdominal wall on both sides of the midline.

FINDINGS

Gross Pathology.—

The new and used airbrasive powder was localized in grayish, flat, irregular masses, mostly on the peritoneal surface along the lower midline of the abdominal cavity (Fig. 1). Some of these gray spots were also found in the mesenteric fat. The pigmented masses were dense to palpation and firmly adherent to the underlying and surrounding tissues. No inflammatory halo could be seen around them. The site of the injection was not visible grossly.

In the animals that had received injections of dentin and enamel powder, a light yellowish-brown pigment could be observed in the same locations as described for the airbrasive material. These areas appeared as diffuse serosal thickenings of the lower abdominal peritoneal surface, slightly firmer than the surrounding tissues, irregular and patchy in distribution. A few slightly pigmented spots were also observed on the omental surface.

Fig. 2.

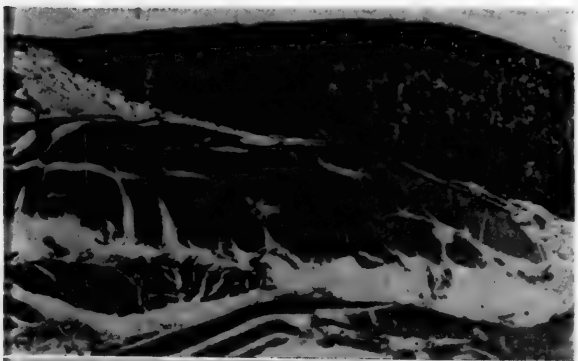


Fig. 3.

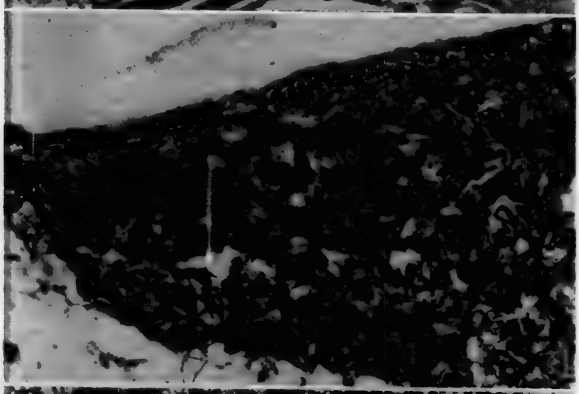


Fig. 4.

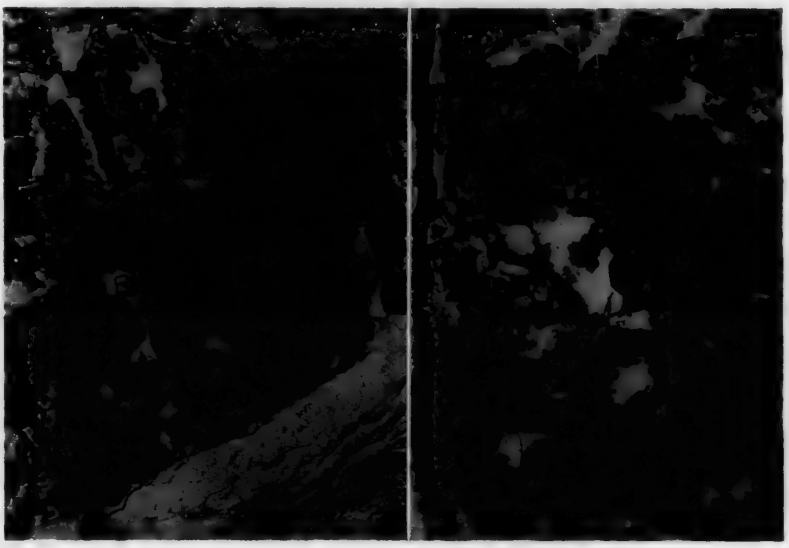
Fig. 2.—Section through a peritoneal plaque of airbrasive powder. (Orig. mag. ×15.)
Fig. 3.—Fibrosis on peritoneal surface in reaction to airbrasive powder. (Orig. mag. ×252.)
Fig. 4.—Mature connective tissue surrounding airbrasive particles. (Orig. mag. ×217.)

Microscopic Findings.—

A. *New and used airbrasive powder:* It was hardly possible to prepare artifact-free thin sections of the specimens containing airbrasive powder due to movement of the large particles during cutting, but adequate sections for detail studies were obtained.

The findings for the new and used airbrasive powder were essentially similar except that a few opaque particles without crystalline structure, thought to be amalgam, were present in the used powder only; but these particles did not in any way alter the tissue reaction. Therefore, the microscopic findings for the 2 groups will be discussed together.

Focal areas of chronic productive peritonitis and omentitis were observed around the masses of dust (Fig. 2). In the clumps and sheets of airbrasive



A. *B.*

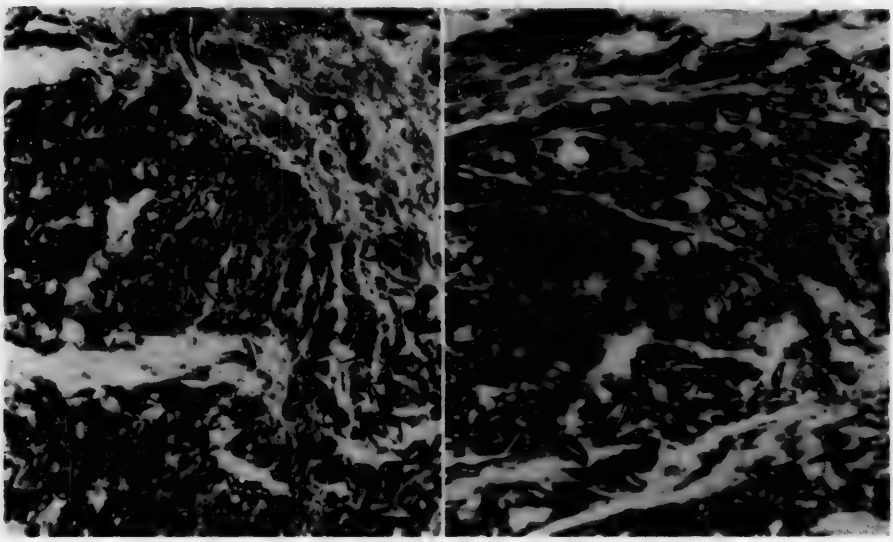
Fig. 5.—*A*, Lymphocytes and plasma cells both in the immediate vicinity of the airbrasive particles and in the surrounding tissues. (Orig. mag. ×406.)

B, High magnification (Orig. mag. ×686) from Fig. 5, *A*. Some particles have a shiny surface; others have dull, granular or black surface. Some particles are smaller than the nuclei of the adjacent cells. Chronic productive inflammation.

powder the individual particles were separated by a large number of proliferating fibroblasts (Fig. 3). Beginning collagen formation was present both at the periphery of the dust aggregations and between some of the dust particles in areas of mature connective tissue (Fig. 4). No necrosis of the type found in typical silicotic granulomas was observed and only a few giant cells were present. Some lymphocytes and a few plasma cells were observed in the immediate vicinity of the particles and occasionally spread rather far into the surrounding tissues (Fig. 5, *A*). The large dust particles had a shiny crystalline appearance, but some of the small particles appeared brown-black (Fig. 5, *B*). A large number of the particles were only 1 to 3 microns in their greatest diameters. It

was difficult to ascertain whether these small particles were phagocytized or situated within the intercellular substances. A large number of cells had the appearance of macrophages. In some areas these cells were closely grouped together, and the boundaries between the nuclei were indistinct or not visible,

Fig. 6.—Mild myositis in abdominal wall adjacent to deposits of airbrasive powder. (Orig. mag. ×686.)



A. *B.*

Fig. 7.—*A*, Gingival fibrosis associated with deposits of airbrasive powder. From rabbit. Time of in observation 6 months. (Orig. mag. ×329.)
B, Dense, mature connective tissue around airbrasive particles. No foreign body giant cell reaction. From free gingiva of rabbit. Time of observation 1 year. (Orig. mag. ×259.)

giving these clusters of cells the appearance of aggregation giant cells. The amount of cytoplasm varied considerably. Some fine brown-black granular material was definitely phagocytized. This appeared as a very fine pigment within a large number of cells. A similar granular pigment appeared on the surface

Fig. 8

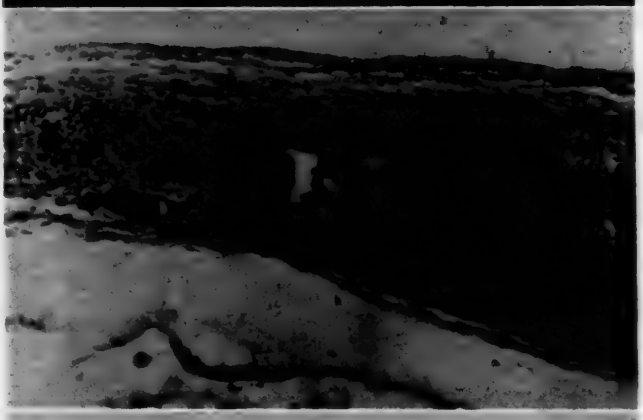


Fig. 9.

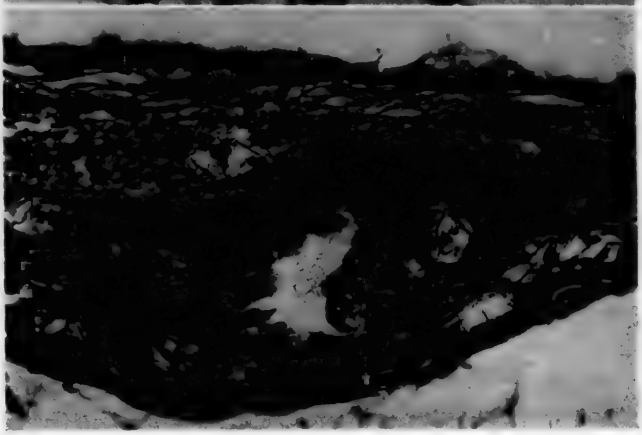


Fig. 10.

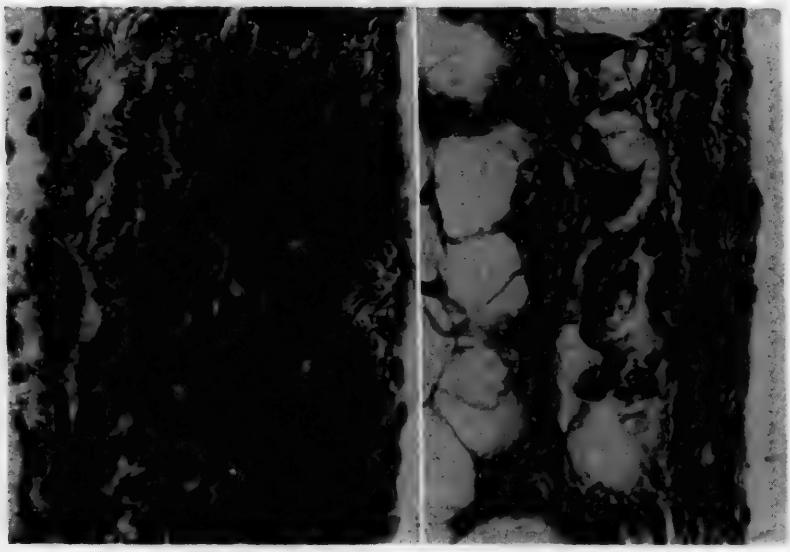
Fig. 8.—Marked foreign body giant cell reaction to enamel dust. Phagocytosis of small particles. Chronic inflammation. Twenty-two day specimen. (Orig. mag. ×686.)

Fig. 9.—Residual dentin dust and mild foreign body giant cell reaction. Fibrosis and chronic productive inflammation. (Orig. mag. ×252.)

Fig. 10.—Collagen formation in an area of peritoneal reaction to dentin dust. Azan stains. (Orig. mag. ×434.)

of some of the dust particles which then had a dull surface in contrast to the shiny surface of the majority of the large crystals (Fig. 5, *B*). No attempt was made to differentiate between the various types of dust particles (Al_2O_3, SiO_2,

A.



B. *C.*

Fig. 11.—*A*, Chronic productive inflammation and a narrow zone of fibrosis on the abdominal serosal surface. Reaction to dentin dust. (Orig. mag. ×217.)

B, Chronic productive omentitis. Fibrosis. Phagocytized granular matter. Reaction to enamel dust. (Orig. mag. ×993.)

C, Small particles phagocytized in large mononuclear cells. Reaction to enamel dust. (Orig. mag. ×993.)

FeO, and CaO) within the tissues. No dust-containing lymph nodes could be found. A slight interstitial myositis was related to some of the masses of air-brasive powder (Fig. 6). No dust or any other abnormalities were observed in sections from the spleen and liver.

The gingival reaction in rabbits to new airbrasive dust was similar to the intraperitoneal findings in the guinea pigs (Fig. 7, *A* and *B*). There was practically no foreign body giant cell reaction, but a rather marked fibrosis. The 1-year specimens showed an increased amount of fibrosis and collagen formation with the production of mature connective tissue as compared to the 6-month specimens. The mucosal membranes were intact over the deposits of airbrasive powder, and practically no inflammatory response could be observed. There seemed to be relatively fewer small particles present in the gingival tissues than in the intraperitoneal specimens. That might have been due either to elimination of the small particles by the body or to deeper penetration of larger particles when the powder was introduced into the gingival tissues.

B. *Enamel and dentin:* The microscopic findings for the enamel and dentin dusts were similar, and they are therefore discussed together.

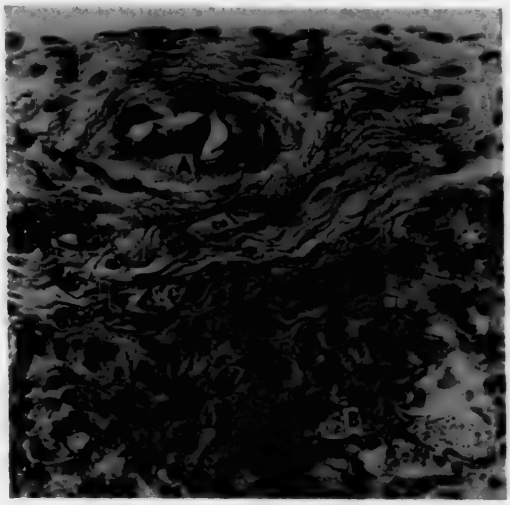


Fig. 12.—Small residual fragment of dentin (*A*) surrounded by fibrous connective tissue without foreign body giant cell reaction. Crystal (*B*) also without foreign body giant cell reaction. Much phagocytized granular material. Chronic inflammation. (Orig. mag. ×993.)

One animal that had received enamel powder was killed by the other animals in the cage after 22 days. A marked foreign body giant cell reaction was then present around the calcified particles (Fig. 8). Phagocytosis of small brown pigmentlike particles and active exudative and productive inflammation was evident in the areas of dust aggregations.

The rest of the specimens were from animals that had 90 days' exposure to the dust. Only occasional areas of a few calcified particles were found with little or no foreign body giant cell reaction around them (Fig. 9). There was a diffuse, mild, chronic productive omentitis on the peritoneal side of the abdominal wall, spread over much larger areas than the reaction to the airbrasive powder. A definite fibrosis could be observed on the serosal surface, evident

also in the connective tissue stains (Fig. 10) as a residual effect of the dust. The fibroblastic activity in the areas of reaction to the dentin and enamel dust was of much milder degree than the reaction to airbrasive dust (Fig. 11, *A*). A large number of large foamy cells contained numerous brownish-black particles of granular pigment (Fig. 11, *B* and *C*). This probably accounted for the light brownish pigmentation seen grossly. A few crystals, 10 to 30 microns in size, were embedded in the serosal surface with very little reaction to them (Fig. 12). These crystals were probably contaminants derived from the ball mill when the dentin and enamel were pulverized. In some of the specimens, a mild chronic myositis was observed in the abdominal musculature related to the areas of surface reaction. Specimens from lymph nodes, spleen, and liver were negative for dust and revealed no significant pathologic changes.

DISCUSSION AND SIGNIFICANCE OF FINDINGS

According to Miller and Sayers,[8, 10] in addition to the usual foreign body reaction, there are 3 types of specific reaction to dust injected intraperitoneally: (1) The proliferative response is characterized by connective tissue nodules progressively increasing in size, gradually becoming firm and fibrotic. (2) The inert reaction occurs when the injected dust neither is absorbed nor initiates a proliferative response. The dust nodules will then become flattened and spread over a larger area. Fine particles are often phagocytized and spread in the surrounding tissues. (3) The absorptive response is characterized by irregular nodules which gradually disappear leaving a small area of fine particles at the site of the nodule. McCord and his co-workers[5, 6] added 2 modified types of response: (A) A mixed response produced by dusts containing some free silica. The reaction is mainly inert, but a few silicotic nodules are found. (B) A miliary proliferative response which is a highly active response with small lesions scattered throughout the abdominal cavity.

It is difficult to classify the reaction to airbrasive powder into any of these specific reactions, but basically the response was slowly proliferative. Whether this proliferative response would ever reach a significant level, as in silicosis, is unknown. The indication is that it would not, since the reaction was so moderate after 90 days. The gingival response after a year was also rather insignificant and more of a scar tissue type than active fibroblastic proliferation. Similar observations were made by Naeslund on mixed silica and aluminum oxide dust.[11] The large crystals seemed unaffected by body fluids. Whether the small aluminum-oxide crystals were attacked and gradually dissolved by the NaCl containing body fluids, as claimed by Jäger and Jäger,[12] cannot be ascertained from this study; but it appeared as if some particles were disintegrating. Neither can it be stated if the proliferative response was due to the aluminum crystals or to the silica which allegedly was present in the powder. It was once believed[13] that the presence of aluminum safeguards against silicosis from the silica-containing dust due to the absorption of a layer of crystalline a-monohydrate of aluminum upon the particles of silica. About 1940-45 an extensive literature appeared on prophylactic and therapeutic use of aluminum dust for

silicosis; but according to the more recent investigators,[14] no definite beneficial effect has ever been proved to follow such practice. Furthermore, combinations of aluminum dust and silica have been found in autopsy cases with severe pulmonary damage.[4] Therefore warnings against the use of aluminum dust for this purpose have been given.[14]

It also has been claimed[12] that the rate of ionization of aluminum and subsequent formation of toxic organic compounds of aluminum-protein salts is accelerated by the presence of mercury, but the old amalgam particles in the used airbrasive did not seem to affect the tissue reaction to the airbrasive powder. There are a few reports in the dental literature on proliferative inflammation around crystalline particles in the gingiva.[15-19] In one case,[19] the particles were proved to be silica, and in 3 other cases it was assumed that they probably were silica. Kollar, Wentz, and Orban[20] abraded 12 healthy gingival papillae with airbrasive material and found very little inflammatory reaction to the crystals. Their claim that aluminum oxide crystals are inert cannot be accepted as proved since their time of observation, at the most, was 2 days. More significant is that in an area of pulpal exposure with the airbrasive technic, Patterson and Van Huysen[21] did not find any inflammatory reaction to airbrasive particles after 60 days.

When a rotating brush and fine pumice are used for polishing teeth in the presence of gingival ulcerations, it is probable that some silica particles will be forced into the gingival tissues, superficially. Clinically, this does not seem to be of the same significance as, for instance, talcum powder left behind in the peritoneal cavity, probably because very little, if any, silica is incorporated within the gingival tissues following healing of these superficial wounds.

With the proliferative response (evident in this investigation) in mind, it appears that the introduction of airbrasive dust into the oral tissues constitutes an undesirable hazard producing a fibrotic response. Whether the proliferative reaction could be avoided by elimination of the silica from the airbrasive powder is not known.

The reaction to enamel and dentin would basically fall into reaction type III, the absorptive response, although some fibrosis also ensued from these dusts. The resultant mild fibrosis appears to make it undesirable to inhale considerable amounts of dentin and enamel dust as may occur when dentists "dry prepare" dental cavities and subsequently inhale the dust over many years. No inhalation experiments were undertaken for these dusts but, as previously stated, it is well established that the pulmonary changes are similar to the peritoneal changes. Therefore, it is probably advisable for the dentist to breathe through a filtrating mask if he routinely does his dental preparations in a dry field, although it should be emphasized that the reaction to these types of dusts is more of a "nuisance" than frankly irritative.

SUMMARY

1. New and used airbrasive powder initiated a mild proliferative response in the peritoneal cavity of guinea pigs and in gingival tissues of rabbits. It

cannot, therefore, be characterized as being inert, and precautions should be taken against inhaling it or introducing it into oral tissues.

2. Powdered enamel and dentin primarily excited an absorptive response in the intraperitoneal cavity of guinea pigs; but a slight fibrosis also resulted from these dusts. It appears undesirable to inhale them in extensive amounts over many years.

Acknowledgment is made to Miss Kate Mallory for preparing the histologic sections and to Miss Winnifred Arnold for photomicrography.

REFERENCES

1. Gardner, L. U., and Cummings, D. E.: The Reaction to Fine and Medium-Sized Quartz and Aluminum-Oxide Particles. Silicotic Cirrhosis of the Liver, *Am. J. Path.* **9**: 751-63, 1933.
2. Goralewski, G.: Klinische und Tierexperimentelle Studien zum Frage der Aluminumstaublunge, *Arch. f. Gewerbepath u. Gewerbehyg.* **9**: 676-89, 1939.
3. Marchi, A.: Über Experimentelle Aluminumstaublunge, *Schweiz. Ztschr. Tuberk.* **4**: 413-33, 1947.
4. Shaver, C. G., and Riddell, A. R.: Lung Changes With the Manufacture of Alumina Abrasives, *J. Indust. Hyg. & Toxicol.* **29**: 145-57, 1947.
5. McCord, C. P., Fleming, R. L., Ainslee, H., and Johnston, J.: The Measurement of the Harmfulness of Dusts for Humans Through the Agency of Animal Reactions, *Surg., Gynec. & Obst.* **63**: 129-37, 1936.
6. McCord, C. P., Kasper, J. A., and Brosius, W. L.: The Biologic Test for the Determination of the Fibrogenic Properties of Dust, *Ohio State M. J.* **33**: 394-96, 1937.
7. Miller, J. W., and Sayers, R. R.: The Physiological Response of Peritoneal Tissue to Dust Introduced as Foreign Bodies, *U. S. Pub. Health Rep.* **49**: 80, 1934.
8. *Idem:* The Physiological Response of Peritoneal Tissue to Certain Industrial and Pure Mineral Dusts, *U. S. Pub. Health Rep.* **51**: 167-90, 1936.
9. *Idem:* The Response of Peritoneal Tissue to Industrial Dusts, *U. S. Pub. Health Rep.* **56**: 264-72, 1941.
10. *Idem:* Microscopic Appearance of Experimentally Produced Dust Nodules in the Peritoneum, *U. S. Pub. Health Rep.* **50**: 1619, 1935.
11. Naeslund, C.: The Prevention of Silicosis: Experimental Investigations on the Action of Certain Non-silicous Dusts and Silica in the Origin and Development of Silicosis, *J. Indust. Hgy. & Toxicol.* **20**: 1-30, 1940.
12. Jäger, R., and Jäger, F.: Kolloid-Chemisches und Histo-Chemisches zur Frage der Lungenschädigungen durch Aluminumstaub, *Arch. f. Gewerbepath. u. Gewerbehyg.* **11**: 117-30, 1941.
13. Denny, J. J., Robson, W. D., and Irwin, D. A.: The Prevention of Silicosis by Metallic Aluminum, *Canad. M. A. J.* **40**: 213-27, 1939.
14. Brown, E. W., and von Winkle, W.: Present Status of Aluminum in the Therapy and Prophylaxis of Silicosis, *J. A. M. A.* **140**: 1024-29, 1949.
15. Kaletsky, T.: Chronic Granulomatous Inflammation, *Arch. Clin. Oral Path.* **2**: 70-73, 1938.
16. Spalding, G. R., and Orban, B.: Unusual Inflammatory Reaction of the Oral Mucosa, *J. Periodont.* **16**: 127-35, 1945.
17. Orban, B.: Gingival Inclusions, *J. Periodont.* **16**: 16-21, 1945.
18. Kramer, I. R. H.: Siliceous Granulomatosis of the Gingiva, *Brit. D. J.* **91**: 309-13, 1951.
19. Marsland, E. A., and Fox, E. C.: A Case of Ulceration and Pain of the Gingival and Oral Mucosa Associated With the Presence of Silica, *Brit. D. J.* **99**: 115-18, 1955.
20. Kollar, J. A., Wentz, F. M., and Orban, B.: The Reaction of Clinically Normal Gingiva to Abrasive Injury, *J. Periodont.* **26**: 95-98, 1955.
21. Patterson, S. S., and Van Huysen, G.: The Treatment of Pulp Exposure, Oral Surg., Oral. Med. & Oral Path. **7**: 194-206, 1954.

LOCAL EFFECT OF VARIOUS DRUGS ON THE DURATION OF INFILTRATION ANESTHESIA PRODUCED BY DENTAL ANESTHETIC SOLUTIONS

F. P. LUDUENA

Sterling-Winthrop Research Institute, Rensselaer, N. Y.

IT is known that complete or partial dental anesthesia at times persists too long after the operative procedure for which it was needed. The duration of this undesirable residual numbness of lips, cheeks, and, sometimes also, of the tongue may vary depending on the nature of the anesthetic solution used. Efforts toward the solution of this problem have led to the use of short-acting local anesthetics. Another possibility of minimizing this undesirable residual effect would be the acceleration of the disappearance of anesthesia by the injection of the anesthetized area with appropriate solutions. The use of antagonists of the drugs contained in the anesthetic solution suggests itself for this purpose. However, no drugs are known which would produce this effect by directly antagonizing the effect of local anesthetics on nerve conduction. On the other hand, practically all dental anesthetic solutions contain a sympathomimetic vasoconstrictor, and drugs are available which can inhibit the effect of this type of vasoconstrictor.

In this study, the following mechanisms by which the duration of anesthesia could be reduced have been considered: (a) by reduction or abolition of the vasoconstrictor action of the sympathomimetic agent in the dental anesthetic solution. This was accomplished by the use of sympatholytic agents (piperoxan and 1205 I.S.); (b) by the effect of vasodilators (adenosine, adenosine 5-monophosphate, sodium nitrite) acting as physiologic antagonists to the vasoconstrictors in the solution (l-arterenol, epinephrine); and (c) by increasing the diffusion of the active drugs in the local anesthetic solution with an agent such as hyaluronidase. When combined with the other agents, (a) and (b), hyaluronidase would also increase their penetration and the speed of onset of action.

METHODS

Guinea pigs were used in all experiments. In most of them, anesthesia was determined by the method of Bülbring and Wajda.[3] The procedure was as follows: two wheals were raised on the middle line of the guinea pig's back by the intradermal injection of 0.25 c.c. of the undiluted dental anesthetic solution. Twenty minutes later one of the wheals was reinjected intradermally with the experimental solution and the other was either left uninjected or was injected with saline. In some cases other solutions were substituted for saline,

Received for publication July 11, 1956.

when comparisons were made. The two wheals were injected with the same volume of solution which was either 0.25 or 0.5 c.c. Following Bülbring and Wajda's method, in one half of the animals one solution was injected in the anterior wheal and the other solution in the posterior wheal in the middle line, while in the other half of the animals the position was reversed. Sets of 6 stimuli, as described in the original method, were applied to each wheal 10 minutes after the first injection, 10 minutes later, that is, immediately before the reinjection, and every 10 minutes thereafter until the anesthesia disappeared, or up to 4 hours. The injected areas were pricked with a needle stylet (needle gauge No. 22) mounted on a rubber stopper. With the exception of one experiment (Exp. 21, Table I), the dental anesthetic solution used was Cook-Waite's RNL solution whose active ingredients are: Ravocaine* HCl :0.4 per cent; Novocain, (procaine HCl) 2.0 per cent; and Levophed (levo-arterenol), 1:30,000. In 2 sets of experiments, the technic was modified in the following manner: both the dental anesthetic and the second solution were injected subcutaneously on the back middle line. Only one injection of 0.5 c.c. of the dental anesthetic solution was made in each animal and 20 minutes later the same area was injected subcutaneously with 0.5 c.c. of the experimental solution. Skin twitches were provoked as just described. As a control, the same number of guinea pigs were injected with RNL and reinjected with saline or another solution under the same conditions.

The average anesthetic scores were plotted against time on coordinate paper, and the lines best fitting the points were drawn by eye. The time it took for the average anesthetic score to be reduced to ¾, ½, and ¼ of the maximal value (score of 6) was estimated from these lines. The relative effectiveness of the various experimental solutions was determined in this manner. The toxicity of the compounds was determined by intravenous injection in mice. The $LD_{50} \pm$ its standard error was estimated at 24 hours after injection by the method of Miller and Tainter.[9] The irritancy was determined by intradermal injection in rabbits followed by the intravenous injection of trypan blue.[5, 7]

Compounds Used.—The dental anesthetic solutions used were commercial preparations dispensed in standard Carpules. The following drugs dissolved in saline were tested for dental "anestholytic" or "anesthesialytic" activity.†

Sympatholytics: Piperoxan (piperidyl-methyl-benzodioxane, Fourneau 933). Piperoxan HCl was tested alone and in mixed solutions with other drugs. *1205 I.S.* (2-(N-(2-(N-Methyl carbamyl) ethyl) amine methyl)-1,4-benzodioxane), a sympatholytic studied by Marini-Bettolo, Landi-Vittori, and Bovet,[8] was tested alone.

Vasodilators: Sodium nitrite, adenosine, adenosine-5-monophosphate, adenosine triphosphate (ATP), and [1-(4-hydroxyphenyl)-2-isopropyl amino

*Propoxycaine (2-diethylaminoethyl-4-amino-2-propoxybenzoate HCl).

†"Anestholysis" or "anesthesialysis" is defined here, for practical purposes, as the property of reducing the duration and/or the intensity of the anesthetic effect of local anesthetic solutions independently of the mechanism of action. "True anestholysis" would be the "anestholysis" due to a direct antagonism of the local anesthetic agent on the nerve fiber.

ethanol]. *Hyaluronidase,** a preparation containing 150 TR units per milligram, was tested alone and in mixed solutions with other agents.

Miscellaneous: Superinone (Oxyethylated tert. octyl phenol—formaldehyde polymer).

RESULTS AND DISCUSSION

After raising 2 wheals with the RNL solution in preliminary experiments on 8 guinea pigs, one was reinjected with 0.25 per cent saline while the other was not treated. The duration of anesthesia was longer in the untreated wheal but in the saline treated wheal the anesthesia was of sufficient duration to suggest that saline reinjection could be used as control of the injection procedure in a study such as the one we have undertaken.

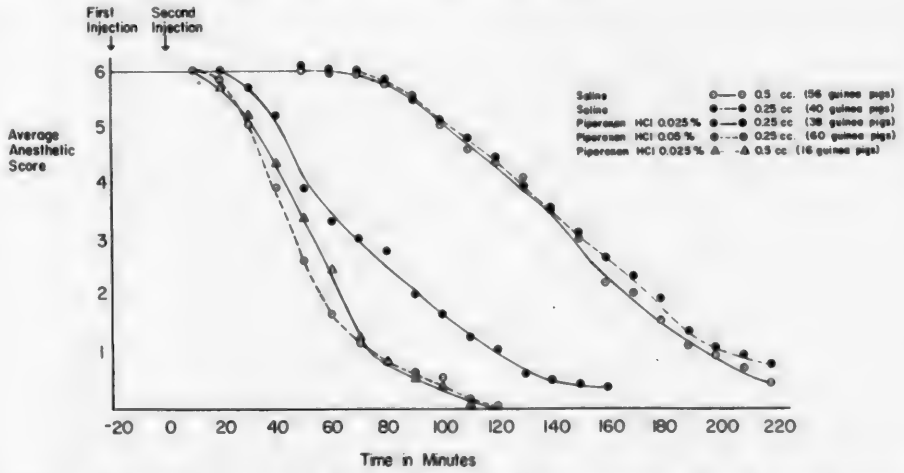


Fig. 1.—Effect of piperoxan HCl on duration of RNL anesthesia. Charts of mean anesthetic scores after reinjection showing the effect of saline, of piperoxan HCl, and the volume injected. The two injections were made intradermally.

Effect of Saline Reinjection.—

There is considerable individual variation in the duration of anesthesia after the injection of the RNL solution and subsequent reinjection (20 minutes later) of saline. Fig. 1 shows the effect of time on the degree of anesthesia of wheals reinjected with 0.5 c.c. of saline, while 40 wheals (5 groups of 8 animals) were reinjected with 0.25 c.c. Obviously the difference in the volume injected had no effect in the duration of anesthesia. There was no change in the maximal score until more than 70 minutes had elapsed after the reinjection. An average value of 50 per cent of the maximal score was reached 150 minutes after reinjection and 25 per cent, 30 minutes later.

Piperoxan HCl.—

This compound was tested in 0.025 per cent, 0.05 per cent, and 0.2 per cent solution. At these concentrations, it reduced considerably the duration

*Delta Chemical Works, Inc.

of anesthesia induced by the RNL solution. With 0.25 c.c. of the 0.025 per cent solution of piperoxan the duration was shortened by approximately 70 minutes (the score-time curves for saline and 0.25 c.c. of 0.025 of piperoxan are approximately parallel). If the concentration or the volume injected is doubled the duration of anesthesia is reduced still further. The effect of 0.25 c.c. of 0.05 per cent piperoxan is about equal to that of 0.5 c.c. of 0.025 per cent. Similar differences occurred also in the time of onset of effects. In the 38 guinea pigs reinjected with 0.25 c.c. of the 0.025 per cent solution all the wheals had complete anesthesia at the 20-minute readings, while in some of the 60 wheals reinjected with 0.25 c.c. of the 0.05 per cent solution or 0.5 c.c. of the 0.025 per cent solution the anesthesia was not complete at this time, indicating that these solutions were producing a measurable effect between 10 and 20 minutes after reinjection.

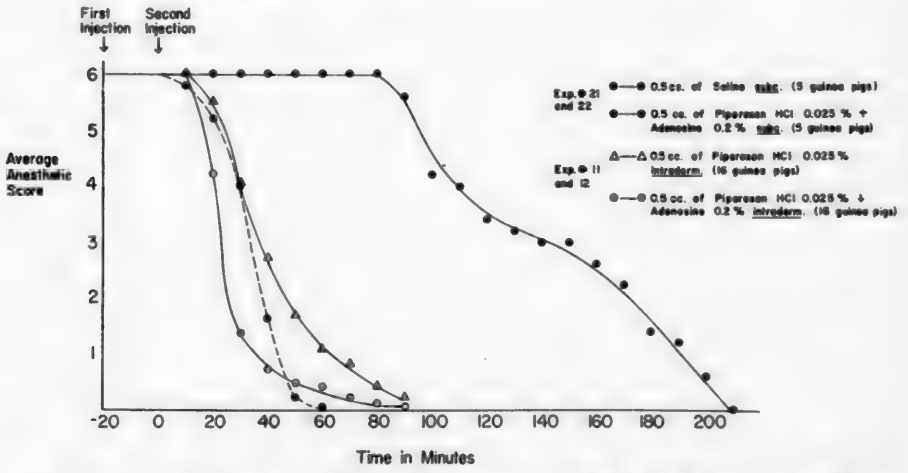

First Injection
Second Injection
Average Anesthetic Score
Time in Minutes
Exp. 21 and 22
Exp. 11 and 12
0.5 cc. of Saline subc. (5 guinea pigs)
0.5 cc. of Piperoxan HCl 0.025 % + Adenosine 0.2 % subc. (5 guinea pigs)
0.5 cc. of Piperoxan HCl 0.025 % intraderm. (16 guinea pigs)
0.5 cc. of Piperoxan HCl 0.025 % + Adenosine 0.2 % intraderm. (16 guinea pigs)

Fig. 2.—Effect of piperoxan HCl and adenosine on duration of RNL anesthesia. Charts of mean anesthetic scores after reinjection showing: (a) the effect of a mixed solution of piperoxan and adenosine after RNL (the two solutions were injected subcutaneously), and (b) the potentiating effect of adenosine (0.2 per cent) on the "anestholytic" effect of piperoxan HCl (0.025 per cent). The local anesthetic and the "anestholytic" solution were injected intradermally in parallel tests).

1205 I.S.—

This compound was tested in comparison with piperoxan HCl, on the duration of RNL anesthesia. When 1 wheal in 16 guinea pigs was injected with 0.25 c.c. of 0.025 per cent of piperoxan and the other wheal with the same volume of 1205 I.S., the effect of piperoxan was more pronounced. In another test on 8 guinea pigs, a concentration of 0.1 per cent of 1205 I.S. was tested in parallel with a solution of 0.025 per cent of piperoxan. The effect was approximately equal, and therefore by this test 1205 I.S. is about one fourth as active as piperoxan in reducing the duration of intradermal anesthesia produced by the RNL solution.

Sodium nitrite.—

Compared side by side with saline, 0.5 per cent sodium nitrite reduced the duration of RNL anesthesia by approximately 50 per cent (Exp. No. 4, Table I). A solution containing 0.1 per cent sodium nitrite and 0.025 per cent piperoxan HCl was more effective than the solution of piperoxan alone (Exp. No. 5).

Adenosine, adenosine-5-phosphate, and adenosine-tri-phosphate (ATP) reduced the duration of RNL anesthesia (Exp. Nos. 6, 7, 8, and 9 in Table I). Although their concentrations were larger they had less effect than 0.025 per cent piperoxan HCl. ATP at 0.1 per cent concentration did not increase the effect of piperoxan HCl (0.025 per cent) (Exp. No. 13). Both adenosine and adenosine-5-monophosphate at 0.2 per cent concentration when mixed with piperoxan had a synergistic effect. The average anesthetic scores of the wheals injected with the mixed solutions obtained at the 20-, 30-, and 40-minute readings were significantly lower than those obtained on the other wheals which had been injected with piperoxan alone (Exp. Nos. 10, 11, and 12).

WIN 833 (levo-1-(4-hydroxyphenol)-2-isopropylaminoethanol bitartrate).—

WIN 833, which is chemically related to N-isopropyl-arterenol and is, like this drug, a vasodepressor[6] had no significant effect at a 0.1 per cent concentration when compared with saline (Exp. No. 14). It had no synergistic effect when added to a solution of piperoxan HCl (Exp. No. 15).

Hyaluronidase.—

At a concentration of 15 TR units per milliliter, hyaluronidase reduced the duration of RNL anesthesia (Exp. No. 16). In Experiment No. 18, the effect of 2 concentrations of hyaluronidase in combination with piperoxan was found to be approximately the same, showing that the smaller concentration was sufficient for maximal effect. At this concentration, hyaluronidase increased the effect of a mixed solution of piperoxan and adenosine. In Experiment 20 was determined the effect of 2 solutions containing piperoxan, adenosine, and hyaluronidase, differing only in the concentration of piperoxan. The solution with the higher concentration of piperoxan was slightly more active.

Miscellaneous.—

Superinone, a nonionic surface-active agent was tested in order to determine whether it increased the speed of diffusion of piperoxan and the active agents in RNL. However, at a concentration of 0.01 per cent in a solution of 0.025 per cent piperoxan it did not modify the effect of this agent, in a parallel test with a 0.025 per cent solution on 8 guinea pigs, as shown by comparison of the average time-score curves of the opposite wheals.

Only in one experiment (6 guinea pigs) a dental anesthetic solution other than RNL was used for the first injection. A solution of piperoxan 0.1 per cent (0.1 c.c.) greatly reduced the duration of anesthesia (Exp. No. 21).

TABLE I

EXPERIMENT	NUMBER OF GUINEA PIGS	FIRST INJECTION 0.25 C.C.	FIRST INJECTION SOLUTION	SECOND INJECTION CONCENTRATION (%)	SECOND INJECTION VOLUME INJECTED (C.C.)	AVERAGE DURATION OF ANESTHESIA AFTER SECOND INJECTION (MINUTES) PER CENT ANESTHESIA 75 (SCORE: 4.5)	50 (SCORE: 3)	25 (SCORE: 1.5)
A	40	RNL	Saline		0.25	118	150	186
B	56	RNL	Saline		0.5	117	150	180
C	38	RNL	Piperoxan HCl	0.025	0.25	47	69	104
D	60	RNL	Piperoxan HCl	0.025	0.50	35	47	63
E	16	RNL	Piperoxan HCl	0.050	0.25	39	54	67
1	8	RNL	1205 I.S.	0.025	0.5	74	83	98
		RNL	Piperoxan HCl	0.025	0.5	44	60	82
2	8	RNL	1205 I.S.	0.025	0.5	92	120	152
		RNL	Piperoxan HCl	0.025	0.5	43	78	108
3	8	RNL	1205 I.S.	0.10	0.5	48	71	90
		RNL	Piperoxan HCl	0.025	0.5	46	59	75
4	8	RNL	Saline		0.25	167	186	236
		RNL	Na Nitrite	0.5	0.25	84	95	130
5	8	RNL	Piperoxan HCl	0.025	0.5	32	44	50
		RNL	Piperoxan HCl Sodium nitrite	0.025 0.10	0.5	25	34	43
6	8	RNL	Saline		0.5	126	156	178
		RNL	Adenosine-5-phosphoric acid	0.2	0.5	82	107	126
7	8	RNL	Saline		0.5	104	136	154
		RNL	Adenosine	0.1	0.5	57	95	125
8	8	RNL	Saline		0.5	103	154	174
		RNL	ATP	0.1	0.5	78	98	110
9	8	RNL	Saline		0.5	108	160	209
		RNL	ATP	0.4	0.5	70	94	124
10	8	RNL	Piperoxan HCl	0.025	0.5	34	46	64
		RNL	Piperoxan HCl + adenosine 5-mono-phosphate	0.025 0.2	0.5	19	27	49
11	8	RNL	Piperoxan HCl	0.025	0.5	34	46	64
		RNL	Piperoxan HCl + adenosine	0.025 0.2	0.5	23	26	30
12	8	RNL	Piperoxan HCl	0.025	0.5	23	30	39
		RNL	Piperoxan HCl + adenosine	0.025 0.2	0.5	15	20	29
13	8	RNL	Piperoxan HCl	0.025	0.5	27	34	47
		RNL	Piperoxan HCl + ATP	0.025 0.10	0.5	29	37	50
14	8	RNL	Saline		0.5	107	132	140
		RNL	WIN 833	0.1	0.5	94	130	157
15	8	RNL	Piperoxan HCl	0.025	0.5	34	51	64
		RNL	Piperoxan HCl + WIN 833	0.025	0.5	37	46	57

TABLE I—CONT'D

EXPERIMENT	NUMBER OF GUINEA PIGS	FIRST INJECTION 0.25 C.C.	FIRST INJECTION SOLUTION	SECOND INJECTION CONCENTRATION (%)	SECOND INJECTION VOLUME INJECTED (C.C.)	AVERAGE DURATION OF ANESTHESIA AFTER SECOND INJECTION (MINUTES) PER CENT ANESTHESIA 75 (SCORE: 4.5)	50 (SCORE: 3)	25 (SCORE: 1.5)
16	6	RNL	Saline		0.25	120	137	175
		RNL	Hyaluronidase	15 U/c.c.	0.25	78	103	126
17	10	RNL	Piperoxan HCl	0.05	0.25	39	54	65
		RNL	Piperoxan HCl + Hyaluronidase	0.05 15 U/c.c.	0.25	33	39	48
18	8	RNL	Piperoxan HCl Hyaluronidase	0.2 15 U/c.c.	0.5	22	29	34
		RNL	Piperoxan HCl Hyaluronidase	0.2 150 U/c.c.	0.5	24	30	50
19	8	RNL	Piperoxan HCl Adenosine	0.05 0.20	0.5	14	28	48
		RNL	Piperoxan HCl Adenosine Hyaluronidase	0.05 0.20 15 U/c.c.	0.5	12	17	26
20	8	RNL	Piperoxan HCl Adenosine Hyaluronidase	0.05 0.20 15 U/c.c.	0.5	16	23	29
		RNL	Piperoxan HCl Adenosine Hyaluronidase	0.2 0.2 15 U/c.c.	0.5	13	18	26
21	6	Novocaine, 2% piperoxan HCl + epinephrine 1:50,000			0.1	95	104	148
		Novocaine, 2% saline + epinephrine 1:50,000		0.1	0.1	40	52	61
		Subcutaneous Anesthesia ENL and antagonists were injected subc.						
22	5	RNL	Saline		0.5	97	140	179
23	5	RNL	Piperoxan HCl Adenosine	0.025 0.20	0.5	26	35	41
24	4	RNL	Piperoxan HCl	0.025	0.5	52	58	67
25	4	RNL	Piperoxan HCl Adenosine	0.025 0.20	0.5	25	32	50

Subcutaneous Injection of RNL and Its Antagonists.—

When RNL and the experimental solutions were injected subcutaneously, it was shown that piperoxan produced a considerable reduction of the duration of anesthesia (Exp. Nos. 22 and 23). The mixture of adenosine-piperoxan was more effective than piperoxan alone (Exp. Nos. 24 and 25).

Toxicity and Irritancy of Piperoxan and Adenosine.—

Mixed solutions of piperoxan and adenosine with and without hyaluronidase were very effective in reducing the length of anesthesia produced by a

dental anesthetic solution. These results suggested that these agents may produce the same effect in clinical dental anesthesia. In order to establish an approximate degree of safety, the toxicity and irritancy of piperoxan was determined. The toxicity was determined by intravenous toxicity in mice. The intravenous LD_{50} of piperoxan HCl and adenosine were: 26 ± 1.8 and > 790 mg./Kg., respectively.

The Threshold Irritant Concentration$_4$ (TIC_4) was determined by the trypan blue method. The TIC_4 of piperoxan and adenosine were: 1.0 per cent and > 1.0 per cent, respectively.

DISCUSSION

The experiments described have demonstrated that the duration of local anesthetics produced by an anesthetic solution containing a sympathomimetic vasoconstrictor can be greatly reduced by various agents. Piperoxan, a fast-acting sympatholytic was the most effective of all the compounds tested. The duration of anesthesia produced by dental anesthetic solutions depended mainly on the vasoconstrictor, and it could be considerably reduced by inhibition of vasoconstriction. Additional vasodilatation by sodium nitrite, adenosine, or adenosine-5-monophosphate shortened the period of anesthesia still further.

The reduction in the duration of anesthesia obtained with adenosine and the adenosine-phosphates was approximately the same, but no attempt was made to determine differences between these compounds by comparing them on parallel tests. Adenosine has been reported as being more dilator than adenosine-5-monophosphate on the vessels of the dog's perfused hind leg.[1]

Inasmuch as adenosine may be constrictor or dilator depending on the type of blood vessels, it is interesting to note that it dilates the rabbit ear vessels[1] and the vessels of the human fingers and toes.[10]

Probably maximal or nearly maximal effects were obtained with piperoxan 0.05 per cent + adenosine 0.2 per cent. With maximal vasodilatation being produced, the time between the reinjection of the wheal and the disappearance of anesthesia represented the time taken for: (a) the diffusion of piperoxan and adenosine within the wheal, and (b) the diffusion of the local anesthetic agents out of the wheal until a value below their combined threshold anesthetic concentration was reached. The addition of hyaluronidase to the solution appears to accelerate this double process of diffusion. However, for practical purposes a piperoxan-adenosine solution may be sufficient for shortening the duration of dental anesthesia. The additional effect of hyaluronidase may not compensate for the problem involved by the instability of the solutions of this enzyme.

WIN 833 failed to change the duration of anesthesia when injected alone or mixed with piperoxan in 0.05 per cent solution. The ratio between this weak vasodilator and that of l-arterenol in the anesthetic solution may have been too low to change the caliber of the vessels. In other words, the effect of l-arterenol may have greatly predominated.

Piperoxan HCl at 0.05 per cent and adenosine at 0.2 per cent are well below the Threshold Irritant Concentration$_4$ (TIC_4) and would not be expected to produce local irritation when injected in dental practice. The toxicity of adenosine is low, and piperoxan has been injected intravenously for diagnostic purposes. It is true that untoward effects (rapid rise of blood pressure, convulsive seizures) had been produced in some patients with essential hypertension with intravenous doses of piperoxan as low as 5 mg.[2, 4] Assuming that the amount of the piperoxan-adenosine solution injected equals the volume of the dental anesthetic solution administered, the maximal total dose of piperoxan would rarely exceed 2 mg. Moreover, this dose would not be administered intravenously.

SUMMARY

1. A series of experiments were carried out on guinea pigs to determine whether the duration of anesthesia produced by the injection of a dental anesthetic solution (Novocain 2.0 per cent; Ravocaine HCl, 0.4 per cent; and Levophed, 1:30,000) could be reduced by the local injection of various agents 20 minutes later. The solutions were injected either intradermally or subcutaneously and the degree and duration of anesthesia was determined by the technic of Bülbring and Wajda.

2. Sympatholytics like piperoxan HCl and 1205 I.S. were effective in reducing the duration of anesthesia produced by RNL, in concentrations as low as 0.025 per cent. Piperoxan was approximately 4 times as active as 1205 I.S.

3. Vasodilators like sodium nitrite (0.5 per cent), adenosine (0.2 per cent), adenosine-5-monophosphate (0.1 to 0.4 per cent) were effective when compared with saline injection.

4. Hyaluronidase (15 TR units per milliliter) was also effective.

5. Of all the compounds tested piperoxan was by far the most effective, showing indirectly the important role played by the vasoconstrictor in the duration of dental anesthesia. Sodium nitrite, adenosine, adenosine-5-monophosphate, and hyaluronidase acted synergistically when injected in mixed solutions with piperoxan.

6. Hyaluronidase increased the effect of a mixed solution of piperoxan and adenosine.

7. The most effective mixed solutions (piperoxan HCl, 0.05-0.2; adenosine, 0.2 per cent; and hyaluronidase, 15 TR units per milliliter) reduced the duration of intradermal anesthesia produced by RNL solution to 10 to 14 per cent of its expected value.

The author is greatly indebted to Dr. J. O. Hoppe and Dr. J. Grant for the toxicity and irritancy data. The excellent technical assistance of Miss P. Bourgault is gratefully acknowledged.

REFERENCES

1. Bennet, D. W., and Drury, N. A.: Further Observations Relating to Physiological Activity of Adenine Compounds, *J. Physiol.* **72**: 288, 1931.
2. Bierman, H. R., and Partridge, J. W.: Untoward Reactions to Tests for Epinephrine-Secreting Tumors (Pheochromocytomas), *New England J. Med.* **244**: 582, 1951.

3. Bülbring, E., and Wajda, I.: Biological Comparison of Local Anesthetics, *J. Pharmacol. & Exper. Therap.* **85**: 78, 1945.
4. Green, D. M., and Peterson, E. M.: Hypertensive Encephalopathy After Administration of Benzodioxan, *J. A. M. A.* **142**: 408, 1950.
5. Hoppe, J. O., Alexander, E. B., and Miller, L. C.: Use of Trypan Blue and Rabbit Eye Tests for Irritation, *J. Am. Pharm. A.* (Scient. Ed.) **39**: 147, 1950.
6. Lands, A. M., Rickards, E. E., Nash, V. L., and Hooper, K. Z.: Pharmacology of Vasodepressor Compounds Structurally Related to Sympathomimetic Amines, *J. Pharmacol. & Exper. Therap.* **89**: 297, 1947.
7. Luduena, F. P., and Hoppe, J. O.: Local Anesthetic Activity, Toxicity, and Irritancy of 2-Alkoxy Analogs of Procaine and Tetracaine, *J. Pharmacol. & Exper. Therap.* **104**: 40, 1952.
8. Marini-Bettolo, G. B., Landi-Vittori, R., and Bovet, D.: Glicinammidi e β-alani-nammidi della del 2-metil-1,4-benzodiossano, *Gazz. chem. ital.* **83**: 144, 1953.
9. Miller, L. C., and Tainter, M. L.: Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper, *Proc. Soc. Exper. Biol. & Med.* **57**: 261, 1944.
10. Vester, A. F.: Untersuchungen über die periphere Wirkung körpereigener Substanzen, *Arch. f. exper. Path. u. Pharmakol.* **166**: 536, 1932.

OBSERVATIONS FROM SHADOWED COLLODION REPLICAS OF TEETH WITH AMALGAM RESTORATIONS

G. T. CHARBENEAU, D.D.S., M.S., AND F. A. PEYTON, D.Sc.

School of Dentistry, University of Michigan, Ann Arbor, Mich.

THE USE of conventional or increased handpiece operating speeds for the shaping of cavities in human teeth results in vibration, temperature changes, and other physical factors within the calcified tooth tissue and its organic matrix. The airbrasive and ultrasonic methods of cutting tooth tissue appear to create similar forces. The effects of these trauma may or may not be significant to the longevity of the healthy functional tooth; this may depend somewhat on the severity of the forces involved.

The ultimate goal of this project is the study of the traumatic effects on both hard and soft tooth tissue which might develop when dental instruments are operated under various conditions. Although the actual development of observable changes in tooth tissue due to rotating instruments is conjecture, it seems likely that some alteration of the normal histologic pattern could occur.

Extracted teeth containing amalgam restorations were selected for study by the shadowed collodion replica technic in order, first, to establish a method of preparation which would allow rapid and accurate examination by the optical microscope, and second, to survey these specimens for any changes from normal which may have occurred. Shadow-casting for optical microscopy, using collodion as the substrate and aluminum as the metallic film,[25] and the general suggestions of Scott and Wyckoff[13, 14] for replica preparation were used as a basis for the development of the technic.

This paper presents in some detail a technic for preparing shadowed collodion replica specimens of sectioned teeth containing restorations, for the general examination of the hard tooth tissue with the optical microscope. Some observations of both normal and affected tooth tissue are presented.

REVIEW OF LITERATURE

Wolf[26] described an "adhesion relief method" for the study of tooth tissue in which celluloid impressions were taken of exposed surfaces. These impressions, which were removed with a sticky cellophane tape, were studied in the optical microscope with transmitted light, usually with oblique relief lighting.

The necessity of having extremely thin specimens in order for investigations to be carried on under the electron microscope[2] resulted in the use of surface replication. According to Zworykin,[28] Mahl formed oxide films on aluminum which produced a negative replica following the removal of the metal. This

This report represents the partial results of studies supported by Contract No. AF 18(600)-427 between the United States Air Force and the University of Michigan.

Received for publication March 30, 1956.

author also described collodion and vinyl lacquer replicating materials for negative replicas and a silver-collodion method for making positive replicas. Other replicating technics and materials have been described in the literature.[1, 4, 5, 11, 12] The topography of the replicas and their interpretation have received comment.[6-8] Richards and Thomassen[10] described the use of the polystyrene-silica replica for the electron microscopic examination of teeth. Gerould[3] also used this method.

Shadow-casting was introduced by Williams and Wyckoff[21] as a procedure for measuring heights of objects on replicas. Shadow-casting was also used as a method of increasing the contrast under the electron microscope, thus producing a three-dimensional, more detailed object.[9, 20, 22-24] It was evident that there was a definite place in optical microscopy for shadow-casting[25]; Scott and Wyckoff[13-15, 18] demonstrated some of its uses in dentistry. Metal shadowed replicas have provided excellent specimens of teeth for electron microscopy also, and numerous reports of these findings have been published.[16-19, 27]

TECHNIC OF SHADOWED REPLICA PREPARATION

From a group of freshly extracted teeth which had been stored in 70 per cent alcohol, an attempt was made to choose a posterior tooth with an amalgam restoration and the remaining tooth structure in reasonably sound condition. A close-up photograph was taken to permit future orientation of the replicas. The tooth was then immersed in 70 per cent alcohol.

1. *Sectioning.*—The tooth was mounted in impression plaster in a square brass block which had a tapered hole through it, approximately ⅝ inch in diameter. The crown was so exposed that the proper sectioning plane was secured. The brass block was screwed tightly in place on a sliding carriage which passed into the path of a disk attached to a lathe engine. The movement of the carriage into the disk and the thickness of the section produced was easily controlled. An 0.013 inch thick aluminum oxide disk* was run at high lathe speed with a constant stream of water directed from each side to the cutting edge. Each section, 1 to 2 mm. thick, was numbered in pencil on the undesired side and returned to its respective bottle.

2. *Polishing.*—Each section was polished on No. 3/0 metallographic polishing paper, then rinsed off; polished on No. 4/0 paper, rinsed off; and finally polished on wet green felt with an aqueous solution of white rouge. The section was thoroughly washed and returned to the specimen bottle. The use of the green felt resulted in some greater selective polishing of the dentin and periphery, and for this reason, may be undesirable for certain studies. It does, however, remove the noticeable scratches remaining from the No. 4/0 paper.

3. *Etching.*—The polished specimen was removed from the 70 per cent alcohol and blotted dry. It was then immersed in 0.05 N HCl for 1 minute, the acid being constantly agitated. At the end of 1 minute, the section was removed from the acid, rinsed in distilled water, blotted dry, and the surface to be replicated dehydrated with 3 or 4 drops of 90 per cent alcohol for 2 minutes, then absolute alcohol for 2 minutes. The sections were then blotted dry and allowed to stand for a few minutes before proceeding. The etching and replication were done in a semi-dustproof enclosure.

*XA-1803-P-RR-5 rubber bonded aluminum oxide wheel, 3-inch diameter with ½ inch arbor hole, 0.013 inch thickness. Allison Co., Bridgeport 8, Conn.

4. *Replication.*—With the surface of the specimen dry, a clear collodion solution was applied with a glass rod. This solution was composed of: 2 parts—flexible collodion, 1 part—amyl acetate, and 1 part—ether. The first clear coat was allowed to dry thoroughly for 15 minutes. At the end of this time, a second coat of the collodion mixture was similarly applied, only to 1 ounce of this solution a small crystal of crystal violet had been added. This stain facilitated the handling of the replica by making it more visible. The second collodion coat was allowed to dry, then the specimen was immersed in cool water. This water immersion appeared to have no detrimental effect on the collodion, and the stripping or peeling of the replica from the tooth section was greatly facilitated. The removal was started by cutting between the replica and tooth with a scalpel, then with microforceps the freed edge was easily grasped.

   The replica was then floated impression side up on a drop of water on a microscope slide which had previously been coated with a thin film of albumin-glycerin fixative. When the slide was placed in an oven at 55° C. for 10 minutes, the replica flattened and became rather firmly attached to the glass.

5. *Shadowing.*—A combination of a mechanical and diffusion pump, capable of pumping down to approximately 5×10^{-5} mm. of mercury, was used to evacuate a large bell jar. An electrode, whose height was adjusted to 16 mm. above the shadowing table, carried a tungsten filament wound to form a basket. Into the basket was placed 1.5 cm. of 24-gauge aluminum wire which was to be vaporized in the high vacuum. The replica was positioned 16 cm. away from the base of the electrode. This resulted in a shadow being cast the same length as the height of the object causing the shadow.

6. *Mounting.*—After the shadow-casting with aluminum, the slides were permanently mounted. A cover slip, 22 × 40 mm., No. 1 thinness, covered the 2 to 3 replicas on each slide. The mounting medium was Harleco synthetic resin 60 per cent in toluene. Weights were placed on the cover slips and the slide placed in an oven for 18 to 24 hours at 55° C.

Although the sections in this report were prepared in the above manner, the use of a cold-cured resin, Ward's Bioplastic, as an embedding medium has since proved useful in supporting weakened tooth specimens, and as an aid in polishing without rounding the periphery of the specimen. After sectioning, the embedded specimens can be polished on 400 and 600 grit silicon carbide paper under a stream of water, then they can be finished on a metallographic polishing wheel with various cloths and abrasives.* This alternate method of polishing allows all work to be carried out in a wet field which provides a more desirable section.

OBSERVATIONS

Since the shadowed collodion replicas are actually negative impressions, the observations become strictly topographical, and the histologist must change his concept of interpretation when viewing the shadowed replica. With this method, shades of gray, produced by the angular shadowing of an irregular surface with metal, are viewed. The technic supplements the conventional histologic preparations, and presents another method of collecting information concerning the hard tooth tissues which may lead to a more complete understanding of their normal histology and pathology. The technic of specimen preparation is simplified, although some of the equipment, namely the vacuum unit, is rather specialized and costly.

*Microcloth (1577AB) with levigated alumina (1557 AB) and kitten-ear broadcloth (1598 S) with levigated shamva (1558 AB). Buehler, Ltd., Evanston, Ill.

The dentin spikes are observed in all sectioning planes, but are more easily demonstrated in the transverse sections (Figs. 1, 2, 3 and 12). There is a zone apparently clear of spikes adjacent to the dentinoenamel junction; this was also observed by Scott and Wyckoff.[15] Although this clear zone decreases in width, it follows around the base of the restorations (Fig. 3). The spikes become more numerous, up to a point, as one moves away from the dentinoenamel junction, then thin out and disappear as the pulp area is approached (Fig. 12). These spikes may be the result of the flow of collodion into the tubules, and since the tubules become smaller in diameter near the dentinoenamel junction, and as sclerosis may occur under caries and restorations, the collodion here may penetrate only a very short distance. The spikes may also result from the removal of some organic material from the tubules when the replica is stripped.

An enamel lamella-like structure (Fig. 4) extends from the dentinoenamel junction to the enamel surface. It is possible that this lamella is a crack produced by the forceps during extraction or during the polishing at the sectioned tooth. In order to alleviate this possibility for the most part, teeth to be extracted for this type of study may be removed with caution and the areas to be studied can be progressively cut, polished, replicated, then recut.[15] This would eliminate the handling of sections even 1.5 to 2.0 mm. in thickness. Embedding of the tooth in the cold-cure plastic would be of value here.

Fig. 5 shows a restoration which parallels enamel rods and Fig. 6 shows one where enamel is unsupported by dentin. Other sections, at both proximal and occlusal margins, have shown distinct undermining and subsequent cracking. It has also been demonstrated that where the dentin apparently is supporting the enamel, a crack has developed nevertheless. Still other sections have shown enamel, unsupported by dentin, with no apparent cracks.

Fig. 7 shows the base of an unfilled fissure which, from gross observation of the tooth section, appeared to have begun decalcification. The dark areas to the right and left of the fissure correspond to the opaque areas on the tooth section. On the replica, these dark areas have received a more even coating of metal; they are smoother then, and apparently were little affected by the etching after polishing. The enamel below the fissure appears coarse. In Fig. 8 the dentinoenamel is shown adjacent to the base of restoration. The enamel near the junction has similar dark areas with coarse enamel running through these. The corresponding areas on the tooth section were opaque.

The enamel immediately below the restoration in Fig. 9 has no rod structure and appears to have been infiltrated by the restorative material. Below this area in Fig. 9 and the enamel immediately adjacent to the restoration in Fig. 10 show again the apparently abnormal coarse arrangement of rods. The observation of this type of enamel adjacent to a restoration was a general finding. This appearance is probably derived from thin vertical projections of the replica, thus casting many shadows (clear area) separated only by a thin dark line (metal coated). The cause and significance of this irregular enamel present a challenging study.

Fig. 1. Fig. 2.

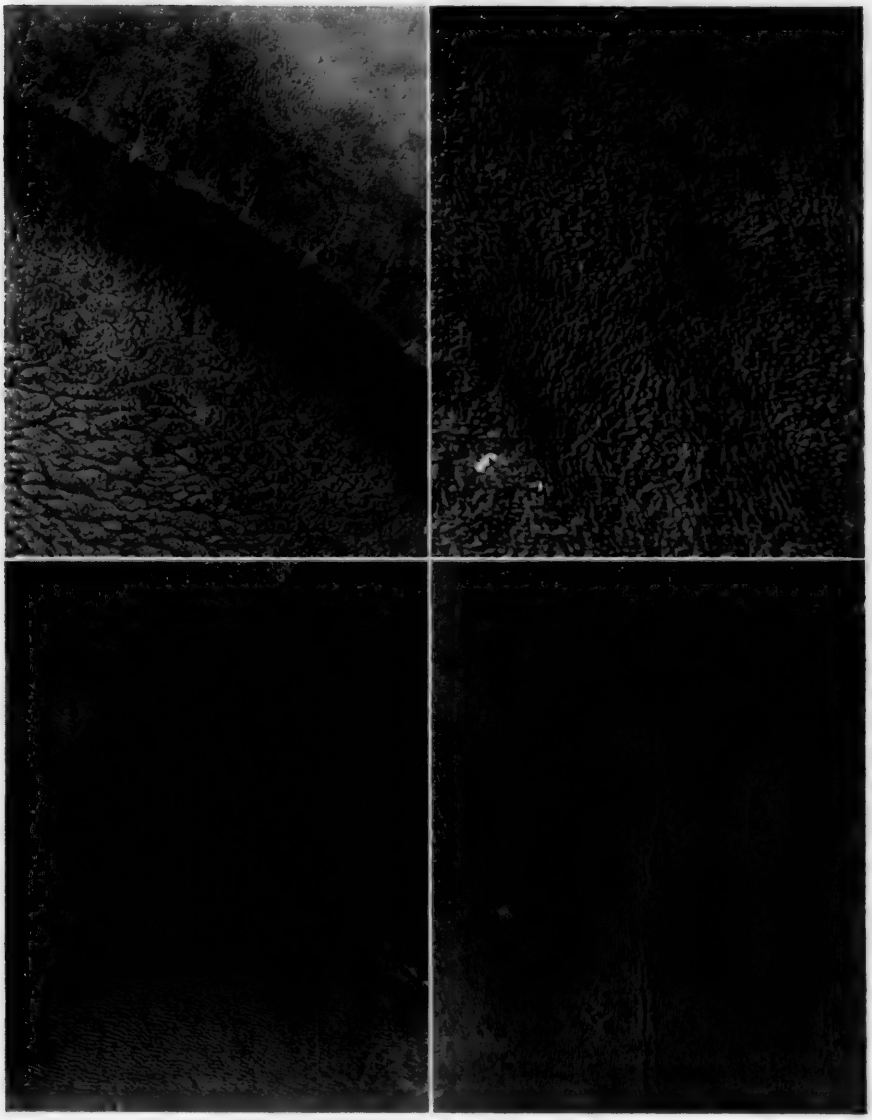


Fig. 3. Fig. 4.

Fig. 1.—Transverse section of mandibular second molar, mesial lingual cusp area. (Orig. mag. ×254.)

Fig. 2.—Transverse section of mandibular second molar, buccal area just above cemento-enamel junction. (Orig. mag. ×378.)

Fig. 3.—Transverse section of mandibular first molar, cervical area of proximal box. (Orig. mag. ×245.)

Fig. 4.—Buccal-lingual section of mandibular first molar, enamel over distal lingual cusp. (Orig. mag. ×245.)

Fig. 5. Fig. 6.

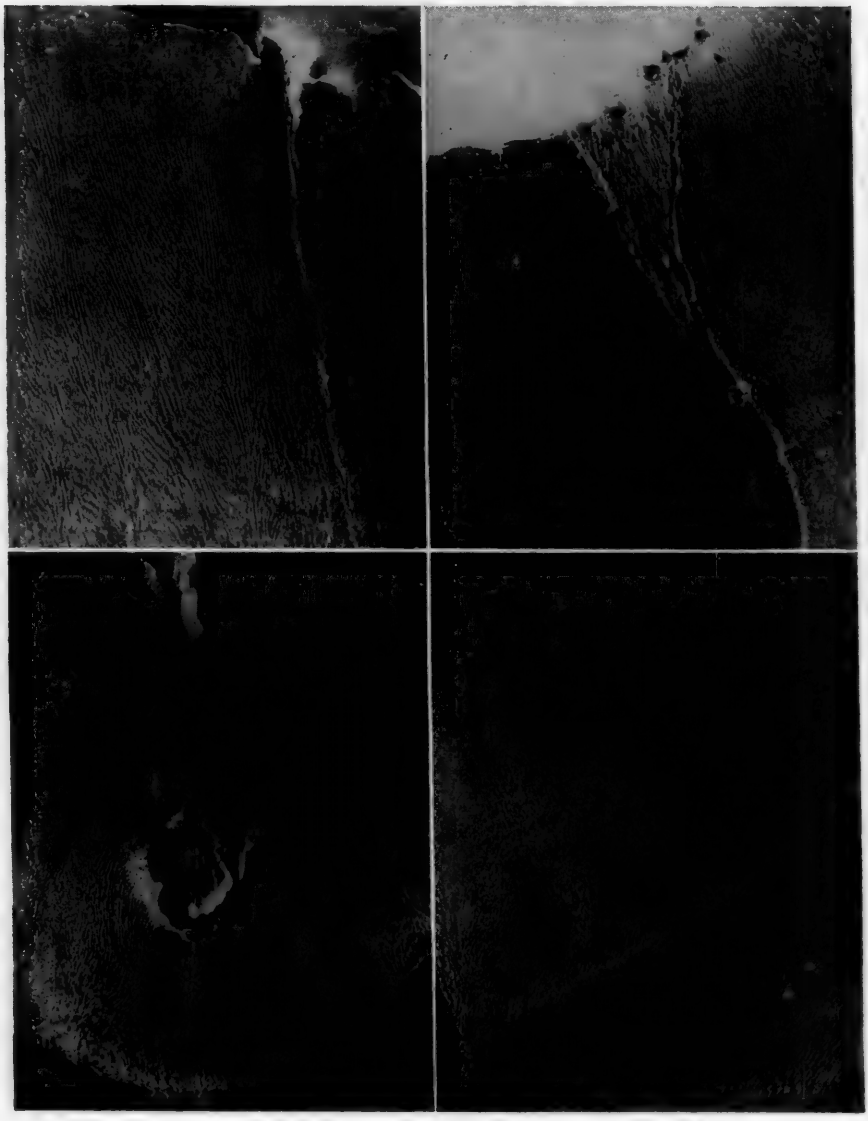


Fig. 7. Fig. 8.

Fig. 5.—Buccal-lingual section of maxillary second premolar with MO restoration through distal aspect of cusps. (Orig. mag. ×245.)

Fig. 6.—Buccal-distal section of maxillary third molar with occlusal restoration, through mesial cusps. (Orig. mag. ×245.)

Fig. 7.—Mesial-distal section of maxillary first molar, through central fossa. (Orig. mag. ×245.)

Fig. 8.—Buccal-lingual section of mandibular second premolar with DO restoration, through mesial fossa. (Orig. mag. ×245.)

Fig. 9. Fig. 10.

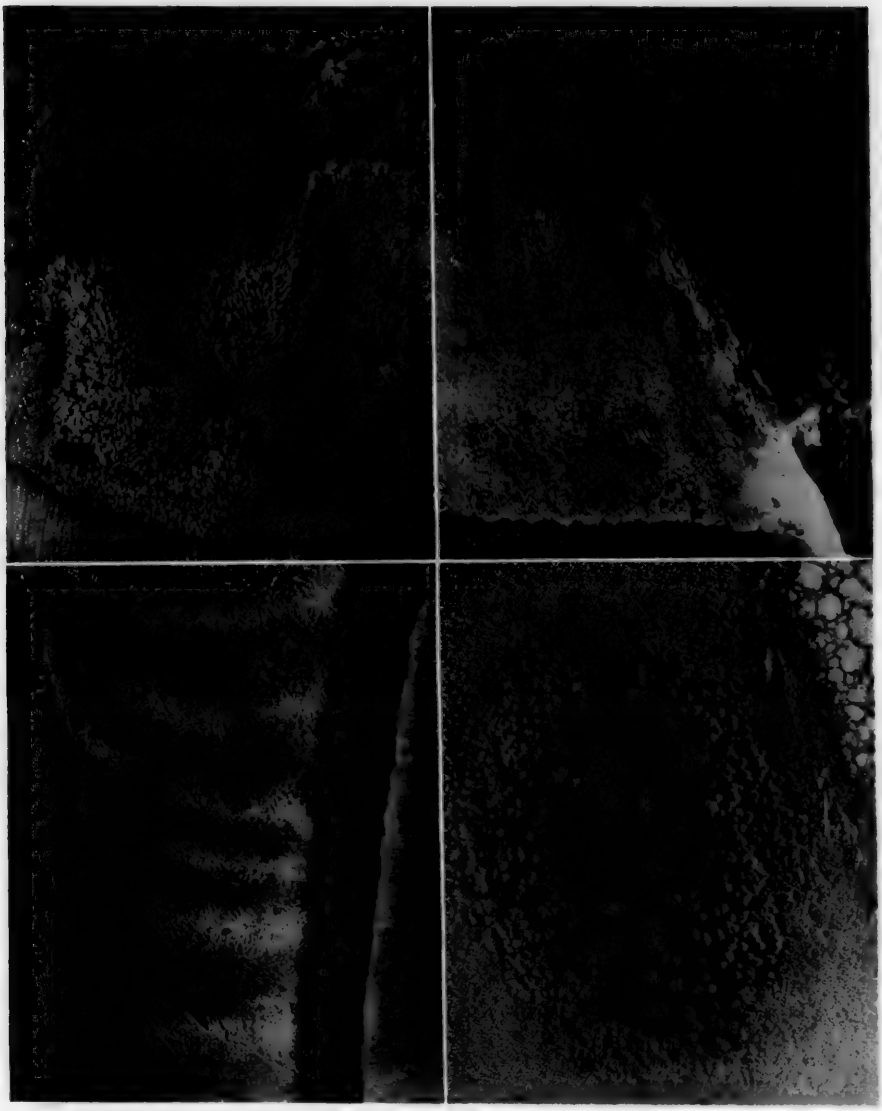


Fig. 11. Fig. 12.

Fig. 9.—Buccal-lingual section of maxillary second molar, through lingual groove. (Orig. mag. ×245.)

Fig. 10.—Mesial-distal section of mandibular second molar with occlusal restoration, through buccal cusps. (Orig. mag. ×245.)

Fig. 11.—Mesial-distal section of maxillary first molar with mesio-occlusal and an occlusal restoration, through buccal cusps. (Orig. mag. ×245.)

Fig. 12.—Transverse section of maxillary second premolar with mesio-occlusal restoration, through cervical floor. (Orig. mag. ×245.)

Hunter-Schreger bands, as observed by this histologic technic, have received considerable comment elsewhere. Fig. 11 shows the Hunter-Schreger bands in parazone and diazone formation running from the dentinoenamel junction to the surface. The dark band at the surface is probably due to a heavy metallic shadowing of that under edge of the replica, since that edge faced the electrode.

In Fig. 12 the adaptation of a cement base is demonstrated in the deep portion of the cavity. This base is separated by a thin band of primary dentin from the secondary dentin which presents an interesting pattern.

SUMMARY

A detailed technic of specimen preparation using the collodion replica method followed by metallic shadowing has been presented. This technic enables one to make rapid and accurate general examinations of the hard tooth tissues under the optical microscope. There are definite advantages gained by studying undecalcified hard tissues, yet the replica eliminates the disadvantages of observation in reflected light or the observation of thin sections with transmitted light.

The use of this technic for the histologic study of tissue surfaces is shown by the photomicrographs taken from the specimens made of teeth with amalgam restorations. The observations demonstrate a need for continued study of the traumatic effects on hard tooth tissue as a result of instrumentation.

REFERENCES

1. Barnes, R. B., Burton, C. J., and Scott, R. G.: Electron Microscopical Replica. Techniques for the Study of Organic Surfaces, *J. Appl. Physiol.* **16**: 730-739, 1945.
2. Burton, C. J., Barnes, R. B., and Rochow, T. G.: The Electron Microscope, *Indust. & Engin. Chem.* **34**: 1429-1436, 1942.
3. Gerould, C. H.: Ultramicrostructures of the Human Tooth as Revealed by the Electron Microscope, *J. D. Res.* **23**: 239-245, 1944.
4. Heidenreich, R. D., and Peck, V. G.: Electron Microscope Study of Surface Structure, *Physiol. Rev.* **62**: 292-293, 1942.
5. *Idem:* Fine Structure of Metallic Surfaces With Electron Microscope, *J. Appl. Physiol.* **14**: 23-29, 1943.
6. Heidenreich, R. D.: Interpretation of Electron Micrographs of Silica Surface Replicas, *J. Appl. Physiol.* **14**: 312-320, 1943.
7. Reidenreich, R. D., and Matheson, L. A.: Electron Micrographic Determination of Surface Elevations and Orientations, *J. Appl. Physiol.* **15**: 423-435, 1944.
8. Picard, R. G., and Duffendack, O. S.: Studies on the Structure of Thin Metallic Films by Means of the Electron Microscope, *J. Appl. Physiol.* **14**: 291-305, 1943.
9. Price, W. C., Williams, R. C., and Wyckoff, R. W. G.: The Electron Micrography of Crystalline Plant Viruses, *Science* **102**: 277-278, 1945.
10. Richards, A. G., and Thomassen, L.: Microstructure of Tooth Surfaces as Revealed by the Electron Microscope, *J. A. D. A.* **31**: 772-776, 1944.
11. Schaefer, V. J., and Harker, D. J.: Surface Replicas for Use in the Electron Microscope, *J. Appl. Physiol.* **13**: 427-433, 1942.
12. Schaefer, V. J.: New Methods for Preparing Surface Replicas for Microscopic Observation, *Physiol. Rev.* **62**: 495-496, 1942.
13. Scott, D. B., and Wyckoff, R. W. G.: Shadowed Replicas of Tooth Surfaces, *Pub. Health Rep.* **61**: 699-700, 1946.
14. *Idem:* Typical Structures on Replicas of Apparently Intact Tooth Surfaces, *Pub. Health Rep.* **61**: 1397-1400, 1946.
15. *Idem:* Shadowed Replicas of Ground Sections Through Teeth, *Pub. Health Rep.* **62**: 422-425, 1947.
16. *Idem:* Electron Microscopy of Tooth Structure by the Shadowed Collodion Replica Method, *Pub. Health Rep.* **62**: 1513-1516, 1947.
17. Scott, D. B., Kaplan, H., and Wyckoff, R. W. G.: Replica Studies of Changes in Tooth Surfaces With Age, *J. D. Res.* **28**: 31-47, 1949.

18. Scott, D. B., and Wyckoff, R. W. G.: Studies of Tooth Surface Structure by Optical and Electron Microscopy, *J. A. D. A.* **39**: 275-282, 1949.
19. *Idem:* Electron Microscopy of Human Dentin, *J. D. Res.* **29**: 556-560, 1950.
20. Thomassen, L., Williams, R. C., and Wyckoff, R. W. G.: Surface Replicas for Electron Microscopy, *Rev. Scient. Instruments* **16**: 155-156, 1945.
21. Williams, R. C., and Wyckoff, R. W. G.: The Thickness of Electron Microscopic Objects, *J. Appl. Physiol.* **15**: 712-716, 1944.
22. *Idem:* Electron Shadow Micrography of the Tobacco Mosaic Virus Protein, *Science* **101**: 594-596, 1945.
23. *Idem:* Shadowed Electron Micrographs of Bacteria, *Proc. Soc. Exper. Biol. & Med.* **59**: 265-270, 1945.
24. *Idem:* Electron Shadow-Micrographs of Haemocyanin Molecules, *Nature* **156**: 68-70, 1945.
25. *Idem:* Applications of Metallic Shadowcasting to Microscopy, *J. Appl. Physiol.* **17**: 23-33, 1946.
26. Wolf, J. Plastische Histologie der Zahngewebe, *Deutsche Zahn-, Mund-und Kieferheilkunde* **7**: 265-284, 507-538, 678-690, 1940.
27. Yasuzumi, G., and Obata, Y.: Electron Microscopy of Human Dentin, *J. D. Res.* **34**: 808-813, 1955.
28. Zworykin, V. K., and Ramberg, E. G.: Surface Studies With Electron Microscope, *J. Appl. Physiol.* **12**: 692-695, 1941.
29. Mortell, J. F., and Peyton, F. A.: Observations of Hunter-Schreger Bands, *J. D. Res.* **35**: 804-813, 1956.

ELECTROLYTIC ACTION IN THE MOUTH AND ITS RELATIONSHIP TO PAIN

J. M. MUMFORD, M.Sc., F.D.S.R.C.S.
University of Liverpool, Liverpool, England

THIS subject has been well reviewed by Schriever and Diamond[1] who also gave a considerable bibliography. However, while it is well known that pain occurs as a result of electrolytic activity in the mouth, it is thought that a clear explanation of how pain occurs has not yet been given. The object of this paper is to try to give such an account.

METHOD

Materials.—Three cylindrical electrodes were made from 22-carat gold, 17-carat gold, and a silver amalgam containing 2 per cent zinc. They were placed, 2 at a time, in a graduated glass tube which had an orifice in its middle portion through which saliva could be introduced (Fig. 1). The saliva was collected from the writer just before each experiment began.

The electrodes, together with their leads to the meter, were coated with wax so that only their opposing surfaces were exposed. These surfaces were ground smooth, but not polished, and cleaned before all experiments by rubbing first on clean fine emery paper and then on clean gauze dental napkins.

Apparatus.—A valve voltmeter with a resistance of 50 megohms was mainly used. When reading in millivolts, the first 2 figures were read off the scale and the third figure was estimated. A direct-reading potentiometer with a standard cadmium cell was used for a few experiments, the results of which were similar to those to be described.

E.M.F. Due to Immersion in Saliva of Silver Amalgam and 22-Carat Gold.—The electrodes were placed with their exposed surfaces 20 mm. apart. Saliva was placed between them to make the cell and electromotive force (E.M.F.) readings were taken at 15-second intervals. Room temperature throughout these experiments varied from 15° C. to 21° C. In an experiment with 1 cell, 30 readings varied only slightly between 563 and 569 millivolts. In all, 25 cells were made for which 257 readings were between 215 and 639 millivolts.

E.M.F. and Interelectrode Distance.—Robbins[2] stated that the greater the distance between the electrodes, the greater the current intensity. Greater distance means greater resistance, so the statement implies an increase in E.M.F. To find if this were so, the E.M.F. was measured as the interelectrode distance was decreased from 20 to 2 mm., the cross sectional area of the

Received for publication Dec. 7, 1955; revised by the author April 23, 1957.

electrolyte being kept constant. The E.M.F. remained approximately constant. For example, in 1 experiment 45 readings were between 562 and 572 millivolts. This suggests that the view expressed by Robbins is incorrect.

E.M.F. and Cross Sectional Area of Electrolyte.—Saliva was added so that the entire face of each electrode was wet and stayed wet as the electrodes were gradually separated. The E.M.F. remained approximately constant although only a thin film of saliva remained. When one electrode was withdrawn from the tube, the E.M.F. was maintained, provided a thin string of saliva clung to it. The E.M.F. is therefore independent of the cross sectional area of electrolyte. This means that if some dentinal tubules are closed by calcium salts, or by dental cements, the E.M.F. will not be affected.

E.M.F., and Electrode Area.—The electrodes and tube were dried and small amounts of saliva were added to increase gradually the electrode areas in the cell. The E.M.F. remained approximately constant.

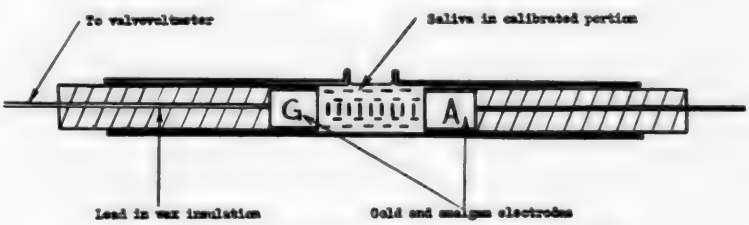


Fig. 1.—Cell with electrodes in position and saliva added.

Effect of Electrodes Coming Into Contact.—The cell was assembled and the E.M.F. noted. Each time the electrodes touched, the E.M.F. fell to zero. When they were separated, an E.M.F. returned but at a reduced value. This was repeated 7 times and each time the E.M.F. was further reduced. The reduction might be due to mercury contaminating the gold.[3]

E.M.F. Due to Immersion in Saliva of Silver Amalgam and 17-Carat Gold, and 22-Carat Gold and 17-Carat Gold.—With silver amalgam and 17-carat gold, 16 cells were made and 296 readings ranged from 180 to 596 millivolts. With 22-carat gold and 17-carat gold, 17 cells were made and 246 readings ranged from 330 millivolts with the 17-carat gold positive to 60 millivolts with the 22-carat gold positive. In one cell the E.M.F. fell to zero and rose in the opposite direction. The smaller E.M.F.'s between the 2 gold electrodes were expected in view of the electrode potentials of the various metals used in making the gold and amalgam.[4]

Current Obtained Through a Microammeter.—A cell with electrodes of silver amalgam and 22-carat gold was connected to a D.C. microammeter with a resistance of 6 kilohms. Readings were made every 15 seconds. In 30 experiments, with various electrode distances the current fell in the same way, rapidly at first and then more slowly (Fig. 2).

Effect of Breaking the Circuit.—Breaking the circuit for 2 minutes permitted the cell to partially recover (Fig. 3), but it did not fully recover even after a 20-minute interval. This incomplete recovery was recognized by

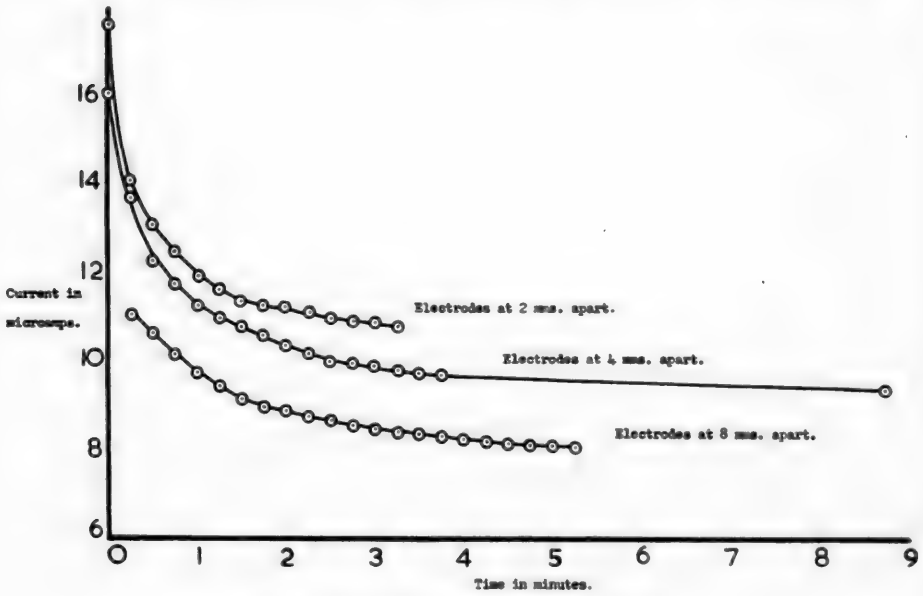


Fig. 2.—Curves showing the fall in current.

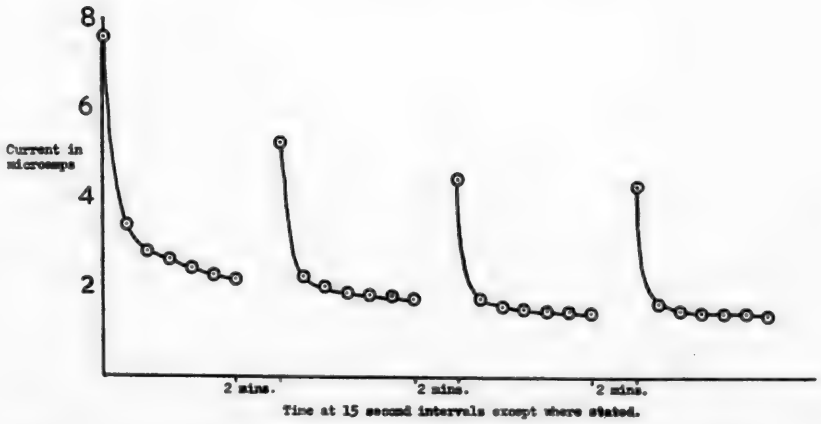


Fig. 3.—Recovery of the cell after breaking the circuit for intervals of 2 minutes.

Reinhard, Solomon, and Goltz[5] who attributed it to desposition of a colloidal film on the electrodes. Electrode "poisoning" in this way is recognized in electrochemistry.[6]

E.M.F. With Electrolytes of Blood and Serum.—Dentin fluid is derived from blood, so blood from a fresh wound and serum from a "blood bank" were used to find what E.M.F. was produced. The results are summarized in Table I. Clotting of blood did not affect the E.M.F., which depends on serum. A cell with serum and electrodes of gold and amalgam gave readings from 200 to 575 millivolts.

TABLE I

COMPARISON OF E.M.F.'S IN MILLIVOLTS

ELECTROLYTE	22-CARAT GOLD AND AMALGAM	17-CARAT GOLD AND AMALGAM	22-CARAT GOLD AND 17-CARAT GOLD
Saliva	470	575	119
Serum	470	545	120
Blood	412	464	40

E.M.F. and Temperature.—The E.M.F. of a cell depends partly on its temperature and this variation (temperature coefficient) is not the same for every cell.[7] Having shown that saliva and serum produced similar E.M.F.'s at room temperature, it was necessary to find if this was so at body temperature. The electrodes and a thermometer were placed in saliva or serum. The cell was slowly heated in a water bath and the E.M.F. was noted as the temperature in the cell rose from 13° C. to 40° C. The average increase in E.M.F. per degree centigrade was expressed as a percentage of the mean E.M.F. The result was 1.64 for saliva and 1.6 for serum. Variation in temperature thus affects the 2 E.M.F.'s similarly.

CURRENTS DUE DO ELECTROLYTIC ACTION IN THE MOUTH AND THEIR RELATIONSHIP TO PAIN

There are several possible electrode systems in the mouth. These are a single electrode, 2 electrodes which remain separated, 2 electrodes which are separated but come into intermittent contact, and 2 electrodes which remain in contact. Combinations of these also occur. The electrodes are usually fillings but one of them may be a metallic denture or any other metal placed in the mouth.

1. *Single Electrode.*—Schriever and Diamond[1] have drawn attention to the possible current due to a single electrode. This has an electrode potential at the saliva surface and another at the bone fluid surface. These, E_S and E_{BF} (Fig. 4), are approximately equal.

E_{LJ} is a liquid junction potential, or diffusion potential, and is due to the transfer of ions across the boundary between the two solutions. In general, according to Glasstone,[8] the larger the ratio of the concentrations of the solutions and the larger the difference between the transference numbers of the two ions, the greater is the liquid junction potential. The sign is derived from the relative magnitudes of the transference numbers of cation and anion of the electrolytes. Compared with the electrode potentials the liquid junction potential is small. The resultant E.M.F. in this case is therefore small, so any current must also be small and unlikely to cause pain.

2. *Electrodes Remaining Separated.*—In this case there is the E.M.F. via the saliva, E_S, and an E.M.F. via the bone fluid, E_{BF} (Fig. 5), and these 2 E.M.F.'s are approximately equal. Schriever[9] regarded these E.M.F.'s as acting against each other so that a possible current would be:

$$\frac{E_S - E_{BF}}{R_S + R_B}$$, where R_S is the resistance through the saliva and R_B the resistance through the bone fluid. The current would therefore be small.

This view would be correct if the 2 electrolytes were quite separate, but the gingiva probably do not afford electrical separation, so there might not be a circuit. In either case pain seems unlikely.

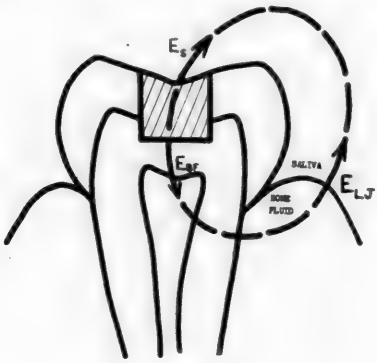


Fig. 4.

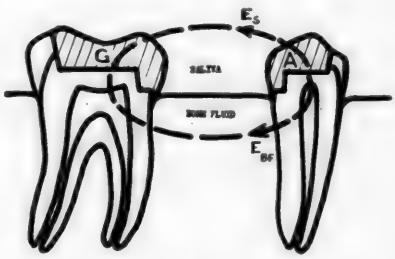


Fig. 5.

Fig. 4.—Single electrode showing the direction of the E.M.F.'s. E_S—Electrode potential of metal with saliva. E_{BF}—Electrode potential of metal with bone fluid. E_{LJ}—Liquid junction potential.

Fig. 5.—Two separated electrodes showing the direction of the E.M.F.'s. E_S—E.M.F. via saliva. E_{BF}—E.M.F. via the bone fluid.

3. *Electrodes Which Are Separated but Which Come Into Intermittent Contact.*—This is the most important group for it is the group in which pain occurs. One of the electrodes must be a filling. The other may be any metal introduced into the mouth, such as another metal filling, a metal denture, metal foil, dental instruments, and domestic cutlery.

In attempting to explain how pain arises the following factors must be considered:

A. *Electromotive force:* Using electrolytes of saliva and serum, E.M.F.'s up to 639 millivolts have been measured. Schoonover and Souder[3] found E.M.F.'s of 445 and 548 millivolts, Schriever and Diamond[1] found E.M.F.'s up to 564 millivolts, and Aasgaard[10] made readings as high as 900 millivolts. Higher E.M.F.'s are possible from other metals, not used as fillings, depending on their position in the electromotive series. The E.M.F. has been observed to fall in value in the first few days after a filling is inserted by Aasgaard[10] and, in a few cases, by the writer. This might be due to "poisoning" of the electrodes by colloidal deposits[5] or to formation of zinc salts in the amalgam leading to a diminution in the quantity of the most

electronegative metal present.[10] The E.M.F. has been shown to fall to zero on bringing the electrodes together and to redevelop on separating the electrodes, but only to a lower value.

B. *Polarization:* If the cell is applied to low resistance such as that of a microammeter (5,000 ohms) it polarizes rapidly.

C. *Resistance of dentin:* This varies considerably, depending mainly upon its thickness. It may be as high as 1 megohm or as low as approximately 10,000 ohms. The surrounding tissues have a relatively low resistance.[11] Björn[12] has shown that the resistance at right angles to the dentinal tubules is 18 times greater than that along them. This is because they contain electrolyte while their walls consist of calcified tissue.

D. *Pain:* When a metal is brought into contact with a metallic filling the patient, sometimes, feels in the tooth a sharp pain which occurs at the instant of contact and lasts for approximately 1 second whether they are immediately separated or remain in contact. If they are separated and then brought together again the pain recurs, but, as the process is repeated, the pain becomes less, and ceases to occur usually after a few times. The pain occurs more readily in newly made fillings. When both electrodes are fillings, pain appears to occur in one tooth and not in the other. Another type of pain that has been ascribed to electrolytic activity is not so clearly related for it is a vague dull pain and takes some time to develop.

HYPOTHESIS TO EXPLAIN THE PRODUCTION OF PAIN BY ELECTROLYTIC ACTION

When the electrodes come together it is supposed that a short circuit occurs giving a relatively high current of abrupt onset. This current has not been measured directly but values may be derived by Ohm's law. The cell is supposed to develop its E.M.F. as a result of the electrodes contacting both saliva and the electrolytes in the dentin and pulp. The E.M.F. is, say, 500 millivolts. The resistance may be anything between 1 megohm and 10,000 ohms. The current would therefore have values ranging from 0.5 to 50 microamperes and it is suggested that the larger of these currents could cause brief sharp pain while lower ones might not. If the metals remain in contact, the current rapidly decreases by polarization and its rate of change is also greatly reduced. The pain would therefore cease.

If the metals are separated, the E.M.F. redevelops, so that a further contact might produce pain in the same manner. This might occur several times, but the E.M.F. gradually falls to such a level that the current produced is insufficient to cause pain. It is suggested that this is the explanation of the clinical observation that pain ceases to occur. The observation that pain seems to occur more with new fillings than with old ones may be explained by the decrease in E.M.F. during the first few days after the filling has been inserted.

The current circuit is shown, in principle, in Fig. 6 but the type of circuit illustrated in Fig. 7 would not produce current that would reach nerves.

When the second electrode is another filling, the circuit may be through both teeth (Fig. 8) or one of the teeth may not be a part of the circuit (Fig. 9). The filling in it would then function as any other piece of metal not actually in a tooth and pain may occur in only one of the two teeth. According to this hypothesis, the pain is more likely to occur in the tooth with the least resistance, but nerve thresholds are presumably also important. In both these instances, the current flows along the dentinal tubules rather than across them because this pathway offers only one eighteenth of the resistance (Figs. 8 and 9).

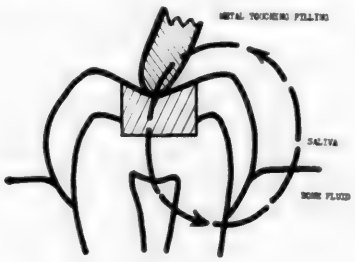


Fig. 6.

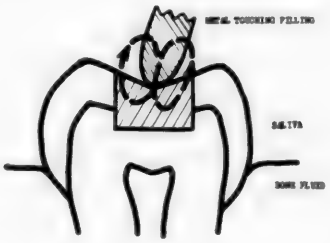


Fig. 7.

Fig. 6.—Direction of current causing pain.
Fig. 7.—Current flowing in this circuit could not cause pain.

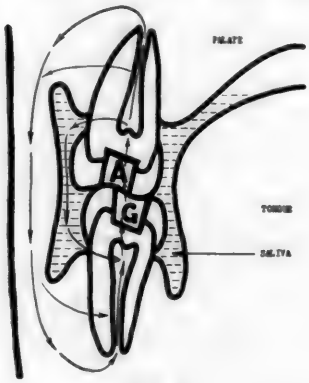


Fig. 8.

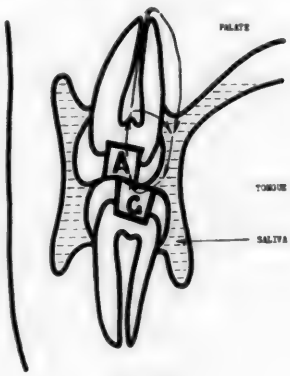


Fig. 9.

Fig. 8.—Pathway of current that might cause pain.
Fig. 9.—Pathway of current more likely to cause pain. The current is regarded as being distributed throughout the tissues in inverse proportion to the electrical resistance.

4. *Electrodes Remaining in Contact.*—Here, there are 2 E.M.F.'s, that produced between the metals with saliva as electrolyte, E_S, and that with the bone fluid as electrolyte, E_{BF} (Fig. 10). A current due to E_S would not reach nerve tissue and therefore could not cause pain. A current due to E_{BF} might cause pain in 2 ways. At the moment when the electrodes first come into contact it is suggested that a short circuit occurs which gives a relatively high current of abrupt onset, and that such a current could cause a brief sharp pain. The

current then falls rapidly, as a result of polarization, to a low, relatively constant level. Such a current would not produce nerve stimulation, and the short sharp pain would not recur. However, the current, continuing for some time, might cause damage to the dental pulp and such damage might be associated with pain of the dull persistent type. This type of pain, if it occurs, is therefore due only indirectly to the current.

The foregoing explanation of the mechanism whereby pain is produced depends mainly on the suggestion that currents of 50 microamperes might cause pain in the teeth. To test this suggestion a current from a dry battery was passed through the teeth and measured by a microammeter in series. The values of the currents which caused pain in 49 patients are given in Table II. Pain occurred with currents comparable to those which it is suggested result from electrolytic action.

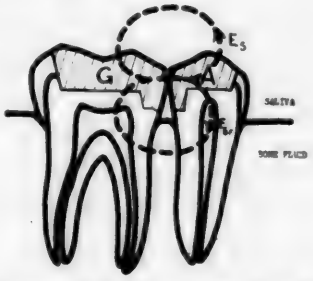


Fig. 10.—Electrodes in contact showing the direction of the E.M.F.'s. E_s—E.M.F. via saliva. E_{BF}—E.M.F. via bone fluid.

TABLE II
CURRENTS AT WHICH PAIN OCCURRED IN THE TEETH

MICROAMPERES PASSING THROUGH THE TOOTH	NUMBER OF CASES
1- 10	2
11- 20	8
21- 30	20
31- 40	6
41- 50	10
51- 60	0
61- 70	1
71- 80	0
81- 90	1
111-120	1
Total	49

SUMMARY

1. Experiments are described in which the E.M.F. was measured when dissimilar dental filling metals were immersed in saliva, serum, and in whole blood. The E.M.F. was similar with each of the 3 electrolytes. Values between 0 and 639 millivolts were obtained.

2. Changing the distance between the electrodes, the area of cross section of the electrolyte, and the area of the electrodes in contact with the electrolyte left the E.M.F. approximately constant.

3. When the electrodes were brought into contact, the E.M.F. fell to zero. When they were separated, the E.M.F. redeveloped but was smaller.

4. Raising the temperature in the cell increased the E.M.F. and the increase was similar for saliva and serum.

5. When the current was measured by a microammeter the cell polarized rapidly. The cell recovered when the circuit was interrupted, but not completely.

6. The results are consistent with those of other workers, and a hypothesis is suggested to explain how pain occurs and why it occurs in some cases and not in others.

REFERENCES

1. Schriever, W., and Diamond, L. E.: Electromotive Forces and Electric Currents Caused by Metallic Dental Fillings, *J. D. Res.* **31**: 205, 1952.
2. Robbins, F.: Electrogalvanic Activity in the Oral Cavity, *Pennsylvania D. J.* **40**: 144, 1938. Quoted by Thoma, K. H., Oral Pathology, St. Louis, 1954, C. V. Mosby Co., p. 959.
3. Schoonover, I. C., and Souder, W.: Corrosion of Dental Alloys, *J. D. Res.* **28**: 1278, 1941.
4. Skinner, E. W.: The Science of Dental Materials, ed. 4, London, 1946, W. B. Saunders Co., p. 456.
5. Reinhard, M. C., Solomon, H. A., and Goltz, H. L.: Further Experiments in Oral Galvanism, *J. A. D. A.* **26**: 1846, 1939.
6. Clark, W. M.: The Determination of Hydrogen Ions, London, 1928, Baillère, Tindall & Cox, p. 438.
7. Findlay, A.: Introduction to Physical Chemistry, ed. 2, London, 1942, Longmans, Green & Co., p. 447.
8. Glasstone, S.: Textbook of Physical Chemistry, London, 1943, Macmillan & Co., Ltd., p. 931.
9. Schriever, W.: Personal communication, 1953.
10. Aasgaard, G.: Personal communication, 1954.
11. Mumford, J. M.: The Relationship Between the Electrical Resistance of Human Teeth and the Presence and Extent of Dental Caries, *Brit. D. J.* **100**: 239, 1956.
12. Björn, H.: Electrical Excitation of Teeth, *Svenska tand. tidning.* **39**: 52, 1946, Supplement.

A NEW PRINCIPLE AND APPLIANCE FOR RADIOGRAPHIC TOOTH MEASUREMENTS*

HARRY H. BENKOW, L.D.S.

Department of Roentgenology, Norwegian State Dental School, Oslo, Norway

DURING the past years many methods for radiographic tooth-length measurements have been published. Most of them are very complicated and lacking in reliability and accuracy. For the general practitioner there is no appliance available the construction of which is simple, the handling easy, and yet the results accurate.

I have recently constructed an appliance for periodic-identical and stereoscopic intraoral radiographs. This has been modified in order to determine the tooth length by means of a single radiograph.[1] The appliance consists of a film-post holder and a distance rod with a focusing ring (Fig. 1). The film-post

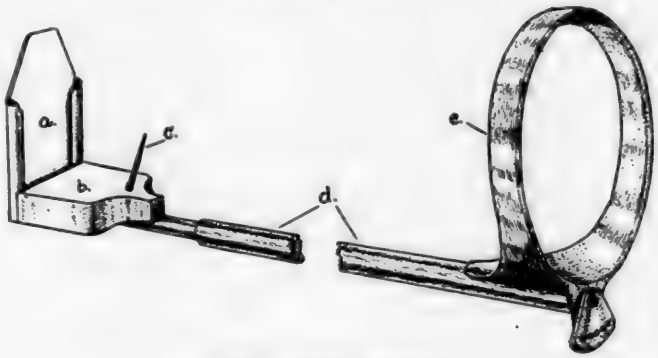


Fig. 1.—The appliance.

holder has a metal backing (a) against which the film rests, and an acrylic block (b) which, at a certain distance from the film, keeps a replaceable steel post (c) forming a fixed angle with the acrylic block. The distance rod (d) gives a film-target distance of 400 mm. (16 inches); consequently, high speed films must be used. There is no firm connection between the focusing ring (e) and the cone. On the latter there are traced 2 lines, parallel with each other and to the base of the cone. The focusing ring must lie close to these lines and its position should be controlled in 2 directions, e.g., from the side and from above.

The true tooth length can now be calculated mathematically by using the following formulas (Fig. 2 shows an outlined drawing of the appliance, tooth, and the target, denoted by letters).

Received for publication July 26, 1956.

*Preliminary report.

(1) $B' = B - \frac{D \cdot a}{D - b - \epsilon}$ (From the triangles pmk and kqs)

(2) $S = \frac{D \cdot a + (D - b) \cdot B'}{D \cdot \sin v + B' \cdot \cos v}$ (From the triangles mnk and ukr)

(3) $T = \sqrt{S^2 - 2S \cdot \epsilon \cos v + \epsilon^2}$

$= S - \epsilon \cos v + \frac{\epsilon^2}{2S} \cdot \sin^2 v + \frac{\epsilon^3}{2S^2} \sin^2 v \cdot \cos v +$ (From the triangle frs)

The appliance is constructed with the following values:

D = 400 mm. (The film-target distance)
a = 22 mm. (The distance between the "central" ray and the acrylic block)
b = 20 mm. (The distance between the film and the post attachment)
v = 68.5 degrees (The angle between the post and the acrylic block).

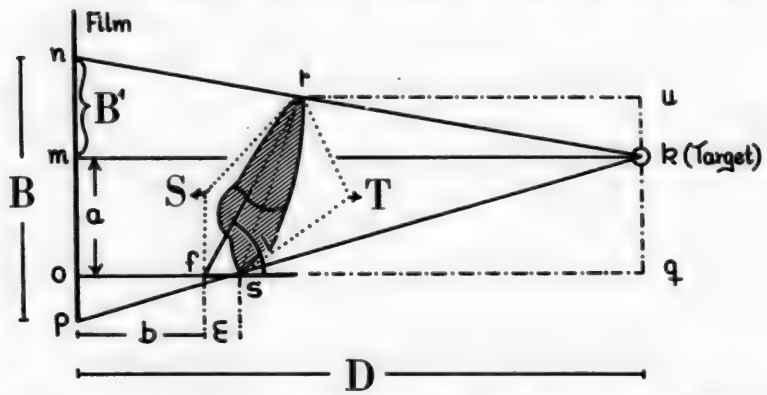


Fig. 2.—Geometric drawing of the appliance. (The proportions are distorted.)

The quantities B and ϵ are also included in the formulas. B = the tooth-image length and is measured directly on the radiograph. ϵ = the distance between the attachment of the post to the acrylic block (Fig. 2, f) and the incisal edge of the tooth (Fig. 2, s) measured on the patient along the upper plane of the acrylic block. Using these 2 measurements and the previously mentioned constant values in the formulas (1), (2), and (3), the true tooth length can be calculated. This procedure, however, will be too complicated and time consuming for the general practitioner. In order to avoid this computation, the tooth length (T) has been calculated in 56 combinations with the B values 10, 15, 20 . . . 45 mm., and ϵ values 0, 1, 2 . . . 6 mm. For each of these combinations the "adjusting" value of the tooth-image length has been computed since $\Delta T = T - B$. The value of ΔT is included in an $\epsilon - B$ diagram and lines are drawn through the points where ΔT has a constant value. These lines are drawn for ΔT = 0.55, 0.45, 0.35 . . . – 2.05 mm. The diagram will then be divided into channels, each with a fixed value for ΔT, with an accuracy of 0.1 mm. (Fig. 3).

When using the appliance the method of procedure is as follows. The tooth is prepared as usual for endodontic treatment. A post (they differ in length and thickness) which fits closely into the canal is chosen. This is attached to the film-post holder which is then put into the mouth, inserting the post in the canal so the incisal edge of the tooth rests against the upper surface of the acrylic block. The distance between the incisal edge and the attachment of the post is noted (ϵ). The distance rod is put on and the patient keeps the appliance in place by finger pressure. The cone of the x-ray machine is placed in the correct position (as earlier described) and exposure takes place. On the developed radiograph the tooth-image length (B) is measured. The point where the lines for the measured values of ϵ and B cross is found on the adjustment

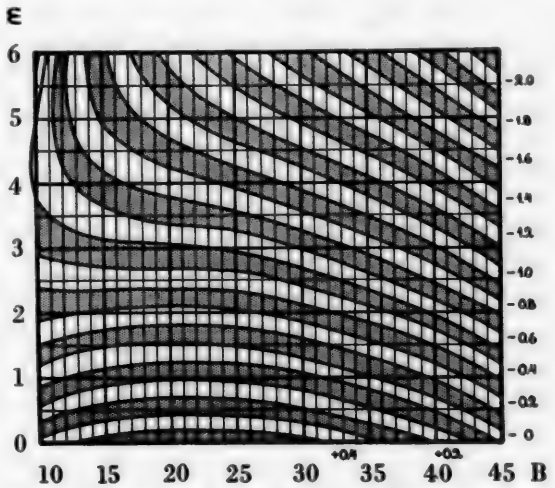


Fig. 3.—Adjustment diagram. Example: The post attachment-incisal edge distance (ϵ) of 3 mm. has been measured. The radiographic tooth-image length is found to be 28 mm. (B). The crossing point for these values lies in the channel with the value of −0.6 mm. The true tooth length is, therefore, 28 mm. − 0.6 mm. = 27.4 mm.

diagram. The value of the channel, where the crossing point lies, is added to the tooth-image length measured on the radiograph (subtracted by minus), and the result is the true tooth length. Slight errors cannot, however, be avoided, because of technical difficulties and because the ideal conditions which were assumed when deducting the formulas cannot always be fulfilled.

At present the method is being tested on extracted teeth as well as on patients (radiographed before extraction) and the results are very good. The error of the method seems to be only slight.

REFERENCE

1. Benkow, H. H.: Periodic-identical and Stereoscopic Intraoral Roentgenograms, *D. Radiog. & Photog.* **30**: 21, 1957.

DIFFERENTIAL GROWTH ANALYSIS OF VERTEBRATE TEETH

MELVIN L. MOSS, D.D.S., PH.D., AND EDMUND APPLEBAUM, D.D.S.

Department of Anatomy, College of Physicians and Surgeons, Columbia University, N. Y.

QUALITATIVE data relating the alterations in size and shape of teeth are commonly known and accepted. Valid quantitative data have been published on vertebrate tooth growth.[18] However, all such material is essentially static. In order to investigate the dynamics of dental ontogenesis we require different methodologies.

A group of recent publications have directed attention to the developmental dynamics of the enamel organ.[1-3, 5, 6, 17] These publications allow a newer interpretation of final crown form in terms of such factors as developmental movements of embryonic tissues, metabolic gradients, and differential growth.

In view of this reactivated interest, we proposed to investigate the differential growth of a series of vertebrate teeth. Such a methodology would yield a more meaningful array of data.[7]

Growth may be defined as the processes which lead to the attainment of adult form. Form may be defined as the resultant of 2 factors: size and shape. During growth, either size or shape, or both, may alter. Indeed, it is a commonplace that vertebrate ontogenesis is characterized by alteration of the relative shape of the whole organism, and of each of its component parts, concomitant with increase in size.

Growth is not haphazard. It is so regulated that the rate of change in any two measurable parameters always exhibits a relative constancy. The method of analysis best suited to provide a simple mathematical expression of this constancy is that of differential growth analysis.

The differential growth equation,

$$(1)\quad y = bx^k$$

may be rendered as,

$$(2)\quad \log y = \log b + k \log x.$$

k is a constant which is the ratio between the specific growth rates of the parameters y and x, i.e.,

$$(3)\quad \frac{dy}{y}\ dt = \frac{d \log y}{dt} = (d \log y = k_1\ dt) \text{ and,}$$

$$(4)\quad \frac{dx}{x}\ dt = \frac{d \log x}{dt} = (d \log x = k_2\ dt)$$

so that

$$(5)\quad k = \frac{k_1}{k_2}\ .$$

The work on which this paper is based was done under the tenure of a Lederle Medical Faculty Award, and aided, in part, by grants, B-965 and A-812, Public Health Service, National Institutes of Health.

Received for publication July 20, 1956.

The term b may be considered merely as the y intercept when the appropriate values are plotted. A more detailed theoretical discussion of the use of this formulation has been presented by Moss.[10, 13]

To graphically illustrate this formulation we need only plot the values of 2 parameters, as y and x, on double log paper. After the line is fitted to these points, the tangent of the angle formed by the line is the numerical value of the constant k. When the angle of the slope is 45 degrees, the tangent value is 1.0 and the ratio of the 2 specific growth rates is unity. This would imply that only size is altering with growth while shape remains constant. If the angle is greater than 45 degrees, the value of k will be greater than 1.0, and the reverse if the angle is less than 45 degrees. In both of these cases, both size and shape will be altering simultaneously, in the first case in favor of the y parameter, in the second case in favor of the x parameter.

On studies of human and rodent ontogenesis of skeletal and neural tissues, it has been shown, in addition, that abrupt changes in the slope of such plots frequently occur. These interphases mark definite stages which correlate with clear-cut histomorphologic changes in the growing tissues.[12-14]

This paper reports the results of such differential growth analysis of a series of vertebrate tooth crowns. It demonstrates the applicability of such a method to this problem.

MATERIALS AND METHODS

The histologic collections of this department contained material suitable for the study of human, rodent, and shark dentitions. Sections of fetal and postnatal human and rodent skulls were viewed with a low-power Leitz projector at a uniform magnification of 30×. Tracings were made and the metric data derived from these tracings.

On sections of decalcified teeth, the width of incisor, premolar, and molar crowns was taken as the greatest distance between the opposing edges of the cervical loop, or its earlier equivalent in the enamel organ as it appeared in fetal sections, or between the opposing edges of the cementoenamel junction in a fully formed tooth. The incisor teeth were studied in labiolingual section, while the posterior teeth were studied in mesiodistal sections. In the incisor teeth, the height of the crown represented the greatest distance of a line at right angles to the line of width, extended to the cuspal summit at the dentinoenamel junction. In the posterior teeth, the height of the highest cusp was measured in similar fashion (Figs. 1 and 2).

RESULTS

Shark.—Fig. 3 is a section of the mandible of a shark (*Carcharodon carcharias*) illustrating the development of the continuously succeeding mandibular dentition. In this section 1 active (erupted) tooth, and 6 reserve (unerupted) teeth are seen. These teeth form a graded series of developmental stages. Plotting the measurements of height and width (Fig. 4) shows that a constant

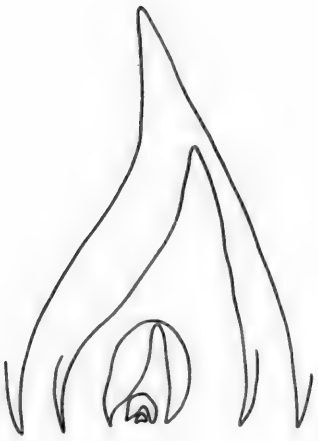


Fig. 1.

Fig. 2.

Fig. 1.—Outline of the dentinoenamel junction of a series of 4 developing human deciduous lower incisor teeth, and a similar outline of a permanent successor. (Orig. mag. ×30.)

Fig. 2.—Outline of the dentinoenamel junction of a series of 3 developing deciduous lower second molars. (Orig. mag. ×30.)

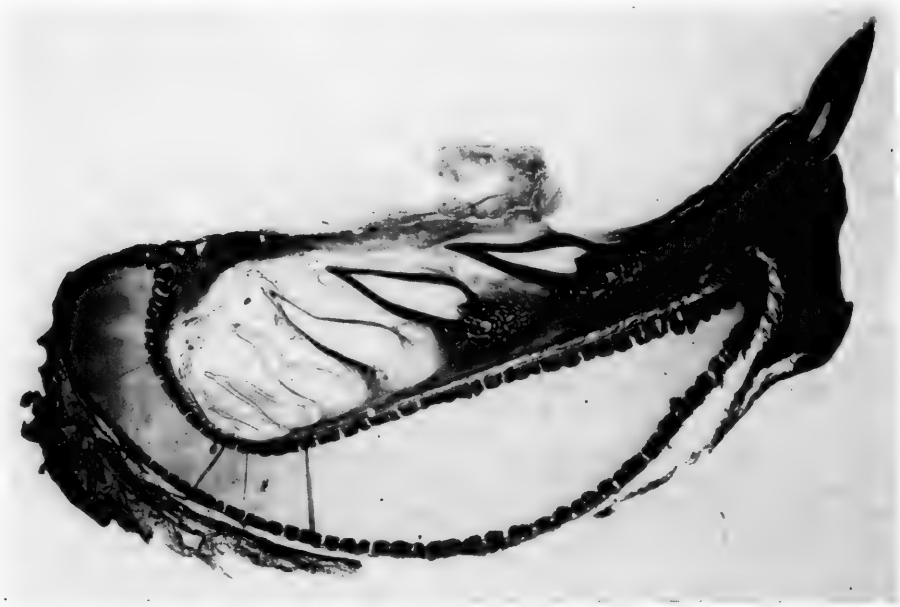


Fig. 3.—The decalcified mandible of a shark illustrating the series of succeeding teeth which were utilized in procuring the data for Fig. 4. (Orig. mag. ×30.)

ratio exists between the specific growth rates of these 2 parameters throughout the developmental stages observed. The numerical value of the tangent of the slope (k = 0.36) indicates that alterations of both size and shape occur continuously during the ontogenetic development leading to the adult definitive tooth.

Rat.—The development of the upper second molar tooth crown was studied in sections of 7 different stages ranging from 2 days prenatal to 35 days postnatal (Fig. 5). It is evident that this plot, as differentiated from Fig. 4, is composed of 2 separate straight lines showing a clear-cut point of flexure (or interphase). This figure indicates that during the first portion of crown formation the specific growth rate of height far exceeds that of width (k = 8.1).

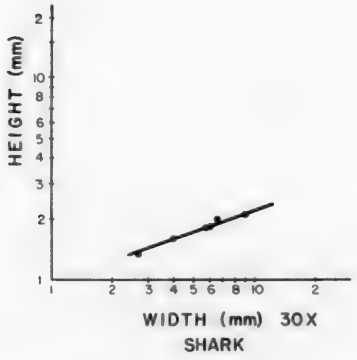


Fig. 4.

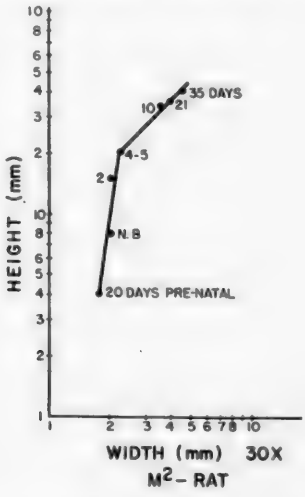


Fig. 5.

Fig. 4.—A plot of the width and height of the dentinoenamel junction outline of the shark teeth illustrated in Fig. 3. Constant relationship exists between the specific growth rates of these 2 dimensions. The slope of the line indicates that size and shape are continuously changing during growth.

Fig. 5.—A plot of the width and height of the dentinoenamel junction outline of the upper second molar of the rat. The numerals give the age in postnatal days, *N.B.* indicates newborn. A sharply defined interphase separates the relative growth curve into 2 distinct phases. During the first phase, size and shape are constantly changing, during the second phase, size alone is altering.

During this period, therefore, tooth crown size and shape were constantly changing. The interphase occurs at approximately the fourth or fifth day. At this stage of development definitive shape is achieved. This is clearly shown in the second portion of the plot. Here the value of the constant (k = 1.00) indicates that only an increase in size occurs, shape remaining constant.

Human.—The lower second deciduous molar tooth crown was studied at 3 stages, at 6-month fetal stage, at birth and at 10 months of age (Figs. 2

and 6). During the stages of growth observed, the developing tooth was constantly changing in both size and shape. The height of the crown increased more rapidly than did mesiodistal width (k = 2.5).

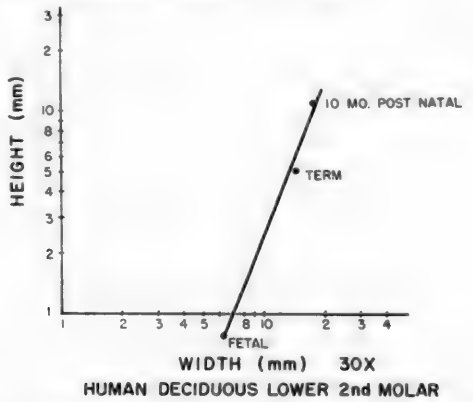


Fig. 6.—A plot of the relative growth of the human deciduous lower second molar. The ages of the specimens are indicated on the figure. During the phase of growth studied, both tooth size and shape are constantly altering.

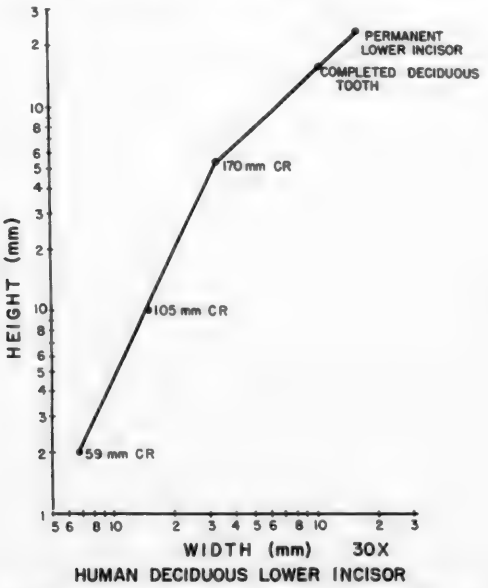


Fig. 7.—A plot of the relative growth of the width and height of the human deciduous lower incisor. The numerals indicate the crown-rump length of the fetus from which the tooth was obtained. Note the interphase, at which time definitive shape is attained, and after which only changes in size occur. The dimensions of the succeeding permanent incisor tooth are relatively the same as the completed deciduous tooth, although absolutely larger.

The lower deciduous incisor crown was studied in a series of 7 sections from a fetus of 59 mm. (crown-rump length) to other sections of the completed tooth. In addition the dimensions of the fully formed permanent successor was also measured (Figs. 1 and 7). This plot also shows a division into

2 separate straight lines, with the occurrence of a sharply defined interphase at the fifth fetal month (170 mm. crown-rump length). During the first portion of ontogenesis, crown height is increasing more rapidly relative to labiolingual width (k = 2.3). The interphase clearly marks the attainment of definitive crown shape, for the second period of ontogenesis is characterized by increase in size alone (k = 1.00). It is interesting to note that the dimensions of the lower permanent first incisor crown are proportionally identical with the deciduous tooth, differing only in absolute size.

DISCUSSION

The results obtained clearly indicate that vertebrate dental ontogenesis is capable of simplified quantitative expression by means of the differential growth equation. The existence of regular and harmoniously integrated patterns of growth of the individual dental organs is quite in accord with the previously ascertained data that such growth patterns exist for human and rodent cephalic growth.[12, 14] Therefore, within the over-all growth pattern of the organism there exists what has been termed a "regressus of patterns."

Our data also indicate that 2 sorts of dental growth patterns exist, (1) that characterized by change in size alone, and (2) that characterized by simultaneous changes in size and shape. During a given period of development these 2 patterns are mutually exclusive, although we have noted that a shift may occur from one pattern to another during the development of a given tooth.

It is quite clear that the type of growth pattern characteristic of a given tooth is not a function of a specific type of dental morphology. That is, incisor teeth do not necessarily possess a simpler growth pattern than premolars. Similar analysis of the data of Gaunt,[5] for mouse dentition, and of Hinrichsen,[6] for the hamster, reinforced our conclusion that there is a marked individuality of growth patterns for each tooth. For example, upper and lower first molar teeth of the mouse possess entirely different patterns of growth. Therefore, we are reasonably accurate in stating that the pattern of dental ontogenesis is highly specific for each tooth, and that no single pattern of dental ontogenesis exists for all the teeth of any one animal. Furthermore, we may state that in the vertebrate series no single pattern exists for teeth of similar morphology or function, i.e., there is no "incisor pattern" which is characteristic of all vertebrate incisors.

Dental morphogenesis: Differential growth analysis is an empirical method. It is useful to us only in so far as it gives a more meaningful interpretation of our data. By itself, it is incapable of making any significant statement concerning the mechanisms of the growth processes. Therefore, in order for this analytic method to have meaning, we must show that it is well correlated with the newer views of the dynamics of tooth formation. We have partially achieved this purpose by illustrating the high degree of coordination which these processes possess. We may now attempt to correlate our data with the histomorphologic aspects of dental ontogenesis.

It may be safely assumed that the final crown form of a tooth has been determined by the alteration of size and shape of the inner enamel epithelium which occurs during growth. The completed tooth crown preserves a record, as it were, of these resultant changes in the morphology of the dentinoenamel junction. According to Butler,[3] the modifications of crown form occasioned by unequal deposition of enamel are only of secondary importance. This same author has recently pointed out that the inner enamel epithelium is normally subjected, throughout its development, to forces imposed by 2 adjacent growing tissues; the stellate reticulum and the mesenchyme of the dental papilla. Butler[3] goes on to state, "On the basal side of the inner enamel epithelium there is the rapidly growing mesenchyme of the dental papilla, and on the occlusal side there is the stellate reticulum, which swells by the accumulation of fluid. The stellate reticulum prevents distortion of the epithelium by growth of the papilla, and thus ensures that folding of the epithelium is due to its intrinsic growth pattern."

Blechschmidt[1, 2] viewed the developing enamel organ from a biochemical point of view. The inner enamel epithelium is felt to be a semipermeable membrane, on either side of which exist metabolic gradients. The well-known histologic and histochemical changes of the cells of this layer are seen as indicating alterations in the dynamic metabolism of the organ. Similarly explained are all of the observed alterations of size and shape of the dental tissues.

The expansion of the crown in any dimension is produced by the active mitosis of the cells of the inner enamel epithelium, with the associated growth of the tissues of the stellate reticulum and of the dental papillary mesenchyme. Cessation of mitosis is characteristic of maturation of cells of the inner enamel epithelium. The formation of odontoblasts and predentin follows the maturation of ameloblasts. Then the ameloblasts begin to produce enamel matrix.[4] The essential point is that any portion of the inner enamel epithelium which has not so matured is capable of growing and so altering either the size or the shape of the crown, or both.

The data obtained by differential growth analysis may aid in two ways in understanding these developmental processes when they are correlated with the histomorphologic data. First, the value of the constant (k) may give a quantitative estimate of either the amount of tissue undergoing mitosis, in a given plane, or a quantitative estimate of the rate of such mitotic activity, which need not be, a priori, equal in all planes. Second, the occurrence of an interphase in the plot clearly indicates a critical developmental horizon in the ontogenesis of the tooth. Such a horizon (or stage) may confidently be investigated with the expectation of observing clear-cut functional changes expressed either biochemically (Needham[15]) or histologically. Such has been the case in our previously published analysis of other organ systems.[10-13] A detailed verification of these theoretical postulates in the case of dental ontogenesis awaits further investigation.

Differential growth analysis is equally valid in the phylogenetic aspects of dental morphology as well as in the ontogenetic. Kurten[8, 9] applied such a methodology to the study of the tooth crown form of bears and concluded that ". . . the study of allometry (differential growth) permits the actual mapping of microevolution events in geologic time."

CONCLUSION

1. The development of crown form in several teeth of the shark, rat, and man was investigated by differential growth analysis.

2. The development of vertebrate crown form was shown by this method to be characterized by two types of patterns, (1) change in size alone, and (2) change in size and shape, simultaneously.

3. The development of a given tooth may entail either one of these patterns exclusively, or both in succession.

4. The type of growth pattern characteristic of a tooth did not appear to be either form or species specific.

5. The theoretical implications of differential growth analysis as applied to dental ontogenesis were discussed.

REFERENCES

1. Blechschmidt, E.: *Acta anat.* **17**: 207-239, 1953.
2. *Idem: Roux Arch. f. Entwicklungsmech.* **147**: 474-488, 1955.
3. Butler, P. B.: *Biol. Rev.* **31**: 37-70, 1956.
4. Diamond, M., and Applebaum, E.: *J. D. Res.* **21**: 403-411, 1942.
5. Gaunt, W. A.: *Acta anat.* **24**: 244-268, 1955.
6. Hinrichsen, K.: *Acta anat.* **23**: 161-180, 1955.
7. Huxley, J.: Problems of Relative Growth, New York, 1932, The Dial Press.
8. Kurten, B.: *Evolution* **9**: 107-118, 1955.
9. *Idem: Acta zool. fenn.* **85**: 1-13, 1954.
10. Moss, M. L.: *Am. J. Phys. Anthrop.* **12**: 71-75, 1954.
11. *Idem: Ann. New York Acad. Sc.* **63**: 528-536, 1955.
12. Moss, M. L., and Baer, M. J.: *Growth* **20**: 107-120, 1956.
13. Moss, M. L., Noback, C. R., and Robertson, G. G.: *Am. J. Anat.* **97**: 155-175, 1955.
14. *Idem: Am. J. Anat.* **98**: 191-204, 1956.
15. Needham, J.: Biochemistry and Morphogenesis, New York, 1950, Cambridge University Press.
16. Noback, C. R., and Moss, M. L.: *J. Comp. Neurol.* **105**: 539-552, 1956.
17. Pruess, F.: *Acta anat.* **18**: 283-295, 1953.
18. Schour, I., and Hoffman, M. M.: *J. D. Res.* **18**: 91-102, 161-175, 1939.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Compiled by KEI ENOKI, *Secretary, Japanese Division*

SCIENTIFIC PROCEEDINGS OF THE JAPANESE DIVISION

1. APPLICATION OF THE PHOTOELASTIC EXPERIMENTS IN THE DOMAIN OF PROSTHETIC DENTISTRY, *Noboru Osada, Hideo Kubota, Takao Miyauchi, Dept. of Prosthetic Dentistry, Tokyo Dental College and Mamoru Yamada, Dept. of Physiology, Faculty of Medicine, Totorri University and Laboratory of Applied Physiology, Tokyo Dental College.* To seek the cause of destruction or fracture of artificial porcelain teeth, photoelastic experiments were conducted on models of artificial incisor, canine, premolar, and molar teeth. All artificial teeth available, commercially, were used as the standard for the models. The investigation was made on the labiolingual or buccolingual and mesiodistal sections of the phenolite model teeth, and the deformation of the denture bases was examined at the same time. The reacting stress was high when pressure was applied from lingual side of anterior teeth. At the same time the compression stresses on the labial ridge and the cervical portion were visible, and extension stresses were found around the retention hole and the alloy pin. The stress changes became greater when the stressed point came nearer to the incisal edge. When pressure was applied to the labial surface at the incisal edge the stress was low. On the posterior teeth high stress was found on the pressure point on the inner inclination of buccal cusp, and compression stress on the buccal side and extension stress around the retention hole and the lingual side were seen. These tendencies were especially apparent on upper premolars. Average compression stress was seen on alveolar surface of the connecting part.

2. PHOTOELASTIC CONSIDERATION UPON DYNAMIC ASPECTS FOR ABUTMENT TEETH OF BRIDGE. *Mamoru Yamada, Dept. of Physiology, Faculty of Medicine, Tottori University and Laboratory of Applied Physiology, Tokyo Dental College. Hirotoshi Shiegeuchi, Masami Homma, and Shohei Homma, Dept. of Dentistry, School of Medicine, Keio University.* A lower first molar, adapted as the abutment tooth of a bridge, was investigated by photoelastic methods. An M-O cavity preparation was made and filled with a metal inlay. The preparation was vertically sectioned to show the maximum mesiodistal width. The surface of this vertical section was used as the original model, and the cervical wall formed an inclination to the inner side. A retention hole was curved in the distal cavity floor. The phenolite plate was ground so that no initial stress was observed. For a second specimen a Chayes type attachment was used. The load was applied and photoelastic photographs were taken. The load for the first specimen was established with 10 Kg. and that for the second specimen

Received for publication April 1, 1956.

with 30 Kg. The cervical wall which has two buccal and lingual bevels and a retention hold increased the stress against the lateral load application. The influence of the degree of bevel of cervical wall is hardly observable. There was more concentration of stress at the buccal cervical bevel and at the neighboring radical side in the movable precision attachment than with the fixed one. The fixed precision attachment showed comparatively stable distribution of stresses.

3. Studies on Dental Calculus by X-ray Analysis. *Kei-ichi Tonogai and Naonosuke Sakuda, Dept. of Pharmacology, Osaka Dental College.* The crystal structure of calculus is an apatite type and the length of its unit cell is a = 9.38A, c = 6.88A, and axial ratio = 0.733. From a crystallographic view, there is little difference in structure of calculus between anterior and posterior teeth. There is no difference of crystal structure of calculus irrespective of metals used for prosthesis or filling. It was observed that calculus generally contains a very small amount of zinc, nickel, strontium, barium, and traces of cobalt, manganese, and ferrum. Calculus deposited on metal surfaces of prostheses or filling contains a small amount of each ingredient of the alloy. A large quantity of copper and zinc has been detected in the gold alloy (Orden), mercury and zinc in amalgam, and zinc in nickel-chromium alloy (Sunplatinum). In the oral cavity, with metal used for prosthesis or filling, a small amount of each ingredient of the alloy has been detected in calculus deposited on teeth having no contact with metals.

4. Physico-Chemical Studies on Salivary Calculus. *Keiichi Tonogai, Dept. of Pharmacology, and Shin-ichi Yoshioka, Dept. of Oral Pathology, Osaka Dental College.* The length of unit cell is a = 9.40A, c = 6.86A, axial ratio = 0.730, and its microcrystal arrangement of an apatite type. There has been detected a small amount of Mg, Al, Fe, Cu, Si, Ba, Sr, Zn, Bi, and Ni in salivary calculus by the spectrum analysis. There has been a small amount of Ba, Sr, Ni, Zn, Fe, Cu, Co and Bi in salivary calculus by the x-ray spectrum analysis.

5. Dentinogenetic Response of the Tooth Pulp to Calcium Hydroxide Application. *Masaru Marushima, Dept. of Operative Dentistry and Dept. of Pharmacology, Tokyo Medical and Dental University, School of Dentistry.* Calcium hydroxide paste was inserted into the pulp tissue of a dog's canine and, after sacrificing the animal, the tooth was fixed in acetone and alcohol. The pulp was carefully separated from the dentin and embedded in paraffin. The distribution of calcium salt diffused into the pulp tissue, the more precise cellular changes and the correlation among the paste, necrotic layer, and newly formed dentin were observed. One half an hour after the operation, necrotic changes were noted in the tissue surrounding the paste. The necrotic layer was ascertained to be formed only at the area where calcium hydroxide had diffused. Calcium carbonate and aragonite granules were observed both in the necrotic layer and in the newly formed hard tissue. The immature calcified tissue originally formed in the pulp gradually developed to dentin within 10 days. New odontoblasts seemed to originate from undifferentiated mesenchymal cells.

SUPPORTING ASSOCIATES*

OF

THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

AND

THE JOURNAL OF DENTAL RESEARCH

The Acralite Company, Inc., New York, N. Y.

Julius Aderer, Inc., New York, N. Y.

L. D. Caulk Company, Milford, Del.

Colgate-Palmolive Company, Jersey City, N. J.

Crescent Manufacturing Company, Chicago, Ill.

Dental Materials' Group, I.A.D.R., Indianapolis, Ind.

Dentists' Supply Company of New York, York, Pa.

Hanau Engineering Company, Inc., Buffalo, N. Y.

J. F. Jelenko & Company, Inc., New York, N. Y.

H. D. Justi & Sons, Inc., Philadelphia, Pa.

Kerr Manufacturing Company, Detroit, Mich.

Precious Metals Research Works, Inc., New York, N. Y.

Procter & Gamble Company, Cincinnati, Ohio

Ticonium Division, Consolidated Metal Products Corp., Albany, N. Y.

The S. S. White Dental Manufacturing Company, Philadelphia, Pa.

*Supporting Associates have made contributions to the Association for support of the JOURNAL. These contributions are in multiples of one hundred dollars.

CONTENTS FOR AUGUST, 1957 (*Cont'd*)

Incidence of Caries at Different Locations on the Teeth. John H. Barr, D.D.S., B.Sc.Dent., Ronald R. Diodati, M.S., and Russell G. Stephens, D.D.S. (Tufts University School of Dental Medicine, Boston, Mass.) ---------- 536

Dental Caries and Dental Care Needs in Baltimore School Children (1955). H. Berton McCauley, D.D.S., and Todd M. Frazier, A.B. (Baltimore City Health Department, Baltimore, Md.) ---------- 546

The Metabolism of Natural and Artificial Fluoridated Waters. Martin J. Wagner and Joseph C. Muhler (Indiana University, Bloomington, Ind.) ---------- 552

The Effects of Salivariadenectomy on the Reproductive Organs of the Female Rat. David Bixler, Ph.D., Richard C. Webster, Ph.D., and Joseph C. Muhler, D.D.S., Ph.D. (Indiana University, Bloomington, Ind.) ---------- 559

The Effect of Testosterone, Thyroxine, and Cortisone on the Salivary Glands of the Hypophysectomized Rat. David Bixler, Ph.D., Richard C. Webster, Ph.D., and Joseph C. Muhler, D.D.S., Ph.D. (Indiana University, Bloomington, Ind.) ---------- 566

The Relationship Between the Histology of the Thyroid and the Salivary Glands and the Incidence of Dental Caries in the Rat. David Bixler, Ph.D., Joseph C. Muhler, D.D.S., Ph.D., and William G. Shafer, D.D.S., M.S. (Indiana University School of Dentistry, Bloomington and Indianapolis, Ind.) ---------- 571

Enlargement of Submandibular Salivary Glands of Rats Restricted to Acidic and Basic Solutions as the Sole Source of Drinking Fluid. Gerald J. Cox, Willard G. Clements, Edward C. Hilger, Jr., and David W. DeGrange (School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.) ---------- 576

Oxidative Enzymes. I. Succinic Dehydrogenase, DPN Cytochrome *c* Reductase, Cytochrome Oxidase, and Catalase in Oral, Liver, and Brain Cortex Tissues. Bertram Eichel and Arnold A. Swanson. With the Technical Assistance of Airman Third Class Tommie Bradley (Departments of Experimental Dentistry and Pathology, USAF School of Aviation Medicine, Randolph Air Force Base, Texas) ---------- 581

The Mineralization of Rat Enamel in the Light of Ca^{45} Autoradiography and Microincineration. Leonard F. Bélanger (School of Medicine, University of Ottawa, Ottawa, Canada) ---------- 595

Tissue Response to Airbrasive Powder, to Pulverized Enamel and Dentin. Donald A. Kerr, D.D.S., M.S., Sigurd Ramfjord, L.D.S., Ph.D., and Greta Grape-Ramfjord, L.D.S., M.S. (University of Michigan School of Dentistry, Ann Arbor, Mich.) ---- 602

Local Effect of Various Drugs on the Duration of Infiltration Anesthesia Produced by Dental Anesthetic Solutions. F. P. Luduena (Sterling-Winthrop Research Institute, Rensselaer, N. Y.) ---------- 613

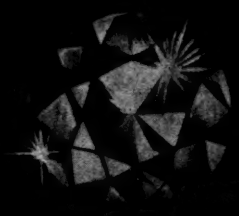
Observations From Shadowed Collodion Replicas of Teeth With Amalgam Restorations. G. T. Charbeneau, D.D.S., M.S., and F. A. Peyton, D.Sc. (University of Michigan, School of Dentistry, Ann Arbor, Mich.) ---------- 623

Electrolytic Action in the Mouth and Its Relationship to Pain. J. M. Mumford, M.Sc., F.D.S.R.C.S. (University of Liverpool, Liverpool, England) ---------- 632

A New Principle and Appliance for Radiographic Tooth Measurements. Harry H. Benkow (Norwegian State Dental School, Oslo, Norway) ---------- 641

Differential Growth Analysis of Vertebrate Teeth. Melvin L. Moss, D.D.S., Ph.D., and Edmund Applebaum, D.D.S. (College of Physicians and Surgeons, Columbia University, N. Y.) ---------- 644

International Association for Dental Research. Scientific Proceedings of the Japanese Division. Compiled by Kei Enoki ---------- 652



STRENGTH FROM STRUCTURE

Although we usually think of pure, crystalized carbon as a decorative gem, the diamond is also widely used for industrial cutting and grinding purposes because of its hardness . . . a prime example of strength from structure.

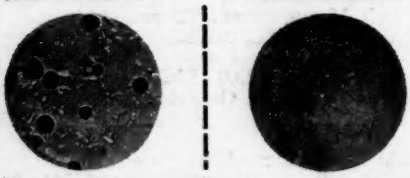
A natural tooth also has built-in strength and beauty, thanks to its internal structure, and man-made teeth should be fabricated from a material with a similar internal structure, if the denture patient is to realize maximum functional and esthetic benefits.

Trubyte Bioform meets this specification! Almost all of the air has been removed in the exclusive Trubyte Bioform vacuum firing process, providing a smooth, dense, homogeneous porcelain which has much of the strength—and is blended to simulate the optical vitality—of healthy natural teeth.

Exact duplication of Nature is not always possible, but Trubyte Bioform Anteriors and Posteriors can honestly be said to "live in the mouth." You can enjoy their many benefits simply by specifying "B For Bioform" Shades on your complete and partial denture cases.

CONVENTIONAL AIR FIRED PORCELAIN

TRUBYTE BIOFORM VACUUM FIRED PORCELAIN



Note in these two photomicrographs how the denser, more homogeneous structure of vacuum fired porcelain is substantially free from the voids and bubbles found in conventional porcelain.

trubyte Bioform® teeth

The VACUUM FIRED PORCELAIN Teeth

THE DENTISTS' SUPPLY COMPANY OF N.Y.

YORK, PENNSYLVANIA